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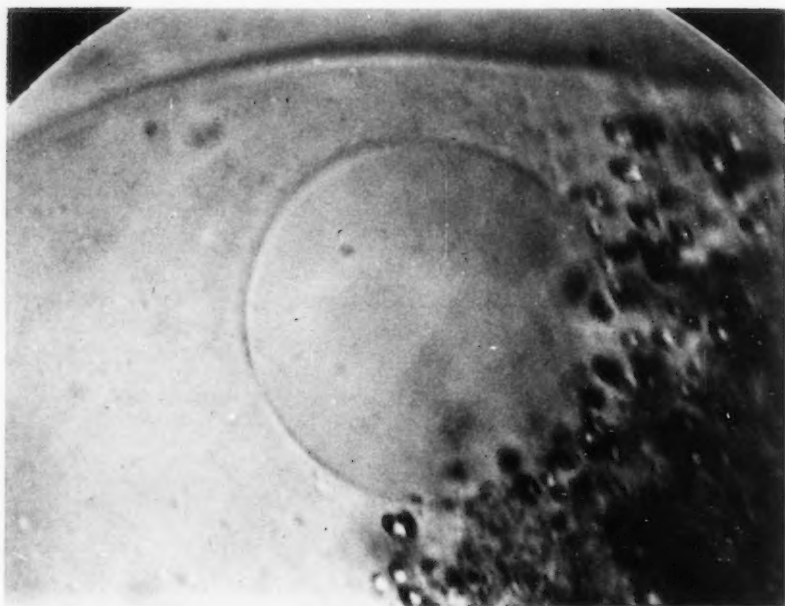


Fig. 1. Contractile Vacuole of Amoeba.



Fig. 2. Crystalline Bodies, Edge of Ectocyst, and Nucleus.

PHOTOMICROGRAPHS OF AMOEBA PROTEUS. OBLIQUE LIGHT, X 1000 DIAMETER.

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NO. 1.

THE RELAXATION OF THE BLADDER MUSCLES OF
THE CAT.

By COLIN C. STEWART.

[From the Laboratory of Physiology of Columbia University at the College of Physicians and Surgeons, New York.]

Relaxation of the detrusors.—Langley¹ has described the effect of stimulating the bladder through its upper lumbar nerve supply as consisting of first a contraction and later a dilation. In a later paper, however, Langley and Anderson² state that they have found no satisfactory evidence of the presence in the hypogastrics of inhibitory fibres. Nor did they find any inhibitory effect on the bladder when it had become contracted in consequence of exposure to the air.

In experiments which have already been reported³ an opposite result has been obtained. Stimulation of the hypogastric nerves with moderately strong induced currents frequently caused a contracted bladder to relax. The usual effect of stimulation with the bladder in a normal condition is exactly that which has been described for it,—a short, and comparatively quick contraction, followed immediately by an apparently active dilation or relaxation of the bladder wall. And the amount of the relaxation is normally much in excess of the amount of the contraction.

In the accompanying figure (Fig. 1) are shown two curves which were obtained by fixing the lower end of the bladder by means of a spike clamped to the same iron standard which carried the lever.

¹ LANGLEY: *Journal of physiology*, 1891, xii, p. xxiii.

² LANGLEY and ANDERSON: *Journal of physiology*, 1895-96, xix, p. 71.

³ STEWART, C. C.: *American journal of physiology*, 1899, ii, p. 182.

The lever, a direct recording one, was attached to the bladder by means of an S-shaped hook caught in its upper end. The first of the two curves shows the normal effect of stimulating the distal cut ends of both hypogastrics together with an induced current of medium strength. The second curve shows the reaction of the bladder to direct stimulation by the same current passed through its entire length for the same time, ten seconds.

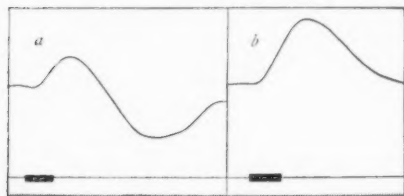


FIGURE 1. One half the original size. Graphic records of (a) the result of stimulating both hypogastric nerves; and (b) the result of stimulating the bladder muscle directly. Time in seconds.

The one is a typical reaction from smooth muscle; the other, however, gives evidence of an active relaxation, both in the time and in the amount of the relaxation.

Figure 2 shows, in one continuous tracing, the curve produced by direct stimulation of the bladder, that given on stimulation of the hypogastric nerves, and, lastly, the effect of stimulating the muscle and the nerves simultaneously. In

the third contraction of the series, as in the second, the relaxation is obviously affected by stimulation of the hypogastric nerves. In other words, stimulation of the hypogastric nerves has either inhibited the contraction produced by direct stimulation, or has in some way produced an active relaxation of the muscle.

The nicotine method of Langley provides us with still another method of demonstrating the inhibitory or relaxing action of impulses carried by the hypogastric nerves.

The course of such impulses is from the lumbar cord through rami, generally three or four on either side, to the inferior mesenteric ganglion. From the ganglion they pass through the two hypogastric nerves, one on either side, to the hypogastric plexus; and from the hypogastric plexus, where the fibres of this upper set meet those arising from the nervi erigentes, the impulses pass by peripheral strands to the bladder itself. On the surface of the bladder the fibres are once more dotted with small ganglia, the ganglia of the vesical plexus.

Thus it may be seen that in the course of fibres passing to the bladder in the upper lumbar supply, there are three possible places where intermediate cell connections may exist: first, in the inferior mesenteric ganglia; second, in the hypogastric plexus; and third,

in the vesical plexus. Accepting Langley's conclusion that in the course of any fibre in the sympathetic system, between its exit from the cord and its peripheral distribution, there is only one intermediate cell connection, we may then represent the anatomical arrangement of the upper group of fibres for the bladder as in the accompanying diagram. (See Fig. 3.)

The fact that there must be a variation in the anatomical arrangement of the fibres and cells leads naturally to the conclusion that a physiological variation may accompany it. That is, if there are fibres of two sorts, it seems possible that those which are concerned

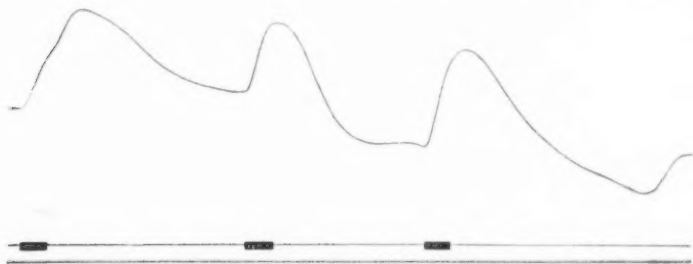


FIGURE 2. One half the original size. Three curves showing the effect of stimulation. First, of the bladder muscle directly; second, of the hypogastric nerves; and third, of both together. Time is indicated in seconds.

in producing contraction alone may have their relay cells in a different ganglion from those which produce dilation alone. Or, if there be no absolute separation, at least the relay cells for dilator fibres will preponderate in one group, while those for motor fibres will be in greater numbers in another situation. If this be true, then the nicotine method lends itself for the demonstration of the separate existence of both. If for example the inferior mesenteric ganglia be painted with a one or two per cent solution of nicotine, such cells as lie in that group will be thrown out of function; and whatever impulses were carried by the fibres in relation with those cells will be blocked. Supposing these cells to be the relays for fibres carrying impulses which produce contraction of the bladder, then after the application of nicotine, stimulation above the ganglia (at *A*) will produce only dilation, or at least chiefly dilation.

Again, if the cells of the second and third groups, — for it is difficult to separate them without interfering with the bladder's blood-

supply—be painted with nicotine, or, better, if from ten to fifty milligrams of nicotine be injected into a vein, then we may expect to poison the relay cells of the dilator fibres. By stimulating the hypogastric nerves (at *C*) after such treatment, the effect ought to be the reverse of that produced by stimulation at *A* in the previous experiment. The result should be chiefly contraction, or possibly contraction alone.

Just such experiments have been carried out in the present research, and with the following results: On painting the inferior

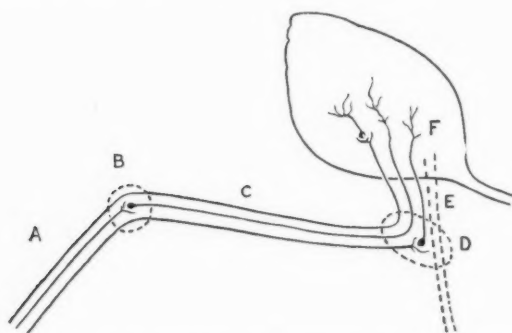


FIGURE 3. A diagrammatic representation of the cell distribution on the course of the fibres in the lumbar supply of the bladder. *A*, the rami to *B*, the inferior mesenteric ganglion; *C*, the hypogastric nerves; *D*, the hypogastric plexus; *E*, the peripheral fibres to *F*, the vesical plexus.

mesenteric ganglia with nicotine, and stimulating the peripheral cut ends of the rami to the ganglia, the result of stimulation is relaxation of the bladder wall; and it is only as the effect of the nicotine gradually wears off that the cells recover sufficiently to transmit impulses causing contraction. The

effect of stimulating was observed by passing a cannula up the urethra, and connecting it with a nearly horizontal manometer upon which variations in bladder volume could be recorded without seriously altering the internal pressure. Graphic records were also obtained by the suspension method already referred to. The result was obtained repeatedly, and with varying amounts of nicotine. With strong solutions of the drug the effect is the same except that recovery does not follow so quickly.

In the experiments of the second group, from 15 to 50 mgm. nicotine were injected into one of the smaller mesenteric veins. As before, the effect of stimulation was observed both by means of a manometer and by the use of graphic records. Stimulation was applied to the peripheral cut ends of both hypogastric nerves together, lasting for 10 seconds. Before the injection of the nicotine

stimulation produced the typical short rise and longer fall of the fluid in the manometer. After the injection the effect is only to produce a rise. Later as the effect of the injection passes off, in a time varying with the amount injected, the relaxation becomes more and more pronounced, until, if the dose of nicotine were not too great, a return to the normal reaction is obtained. Numerous graphic records were also obtained, showing the same effects observed with the manometric method.

Relaxation of the vesical sphincter. — Various experiments have been published to show that stimulation of the sacral supply of the bladder produces a relaxation of the sphincter coincident with the contraction of the detrusor muscles.¹ Against these are the observations of Gianuzzi,² Langley,³ Sherrington,⁴ Griffiths,⁵ and Langley and Anderson.⁶ Mosso and Pellacani⁷ have concluded that the resistance of the sphincter is tonic and constant, and that emission of urine takes place only when the sphincter yields to a pressure sufficiently great to overcome its tonic contraction. And Courtaud and Guyon⁸ have found that although stimulation of the sacral supply relaxes the sphincter, yet the relaxation is a reflex one and does not occur when the peripheral cut ends of the nerves are stimulated. Hanč⁹ has recently demonstrated that the sphincter is independent of the detrusors.

¹ VON ZEISSL: *Ann. mal. org. genito-urinaires*, 1892, p. 828; —: *Archiv f. d. ges. physiol.*, 1893, liii, p. 560.

² GIANUZZI: *Journal de la physiologie*, 1893, vi, p. 22.

³ LANGLEY: *Journal of physiology*, 1891, xii, p. xxiii.

⁴ SHERRINGTON: *Journal of physiology*, 1892, xiii, p. 628.

⁵ GRIFFITHS: *Journal of anatomy and physiology*, 1894-5, xxix, p. 61.

⁶ LANGLEY and ANDERSON: *Journal of physiology*, 1895-96, xix, p. 71.

⁷ MOSSO and PELLACANI: *Archives italiennes de biologie*, 1882, i, p. 291; MOSSO and PELLACANI: *Sulle funzioni della vesica*, 1882; reprinted from *Atti della r. academia dei Lincei*, 1881, series 3, xii.

⁸ COURTAUD and GUYON: *Archives de physiologie*, 1896, xviii, p. 622.

⁹ HANČ, A.: *Archiv f. d. ges. Physiol.*, 1898, lxxiii, p. 453. Dr. Hanč has shown that the rise of pressure brought about by stimulation of the sciatic, as indicated by a manometer in the ureter, bears no constant relation to the amount of emission through the urethra, nor to the latent time of the dilation of the sphincter. The author concludes that there is a reflex dilation of the sphincter (dilator fibres), and supports v. Zeissl's view that the sacral supply contains motor fibres for the detrusors and dilator for the sphincter. His results, however, may equally well be interpreted as showing a reflex inhibition of a tonic sphincter centre. His further experiments in which were studied the effects of various drugs upon the sphincter and detrusor reactions also tend to support this latter interpretation. The depress-

Numerous experiments have been performed during the present research, the results of which tend to confirm the conclusion of Mosso and Pellacani that the resistance of the sphincter is constant. To demonstrate this it is only necessary to connect two cannulas with the bladder in the following way. One, of the usual size for catheterization, is passed up the urethra to a point some distance below the position of the sphincter. The other, of much greater diameter, is inserted at the upper end of the body of the bladder and tied in place. Through the larger tube fluid is expelled from the bladder when its walls contract; and if it be continued upward as a vertical manometer a direct reading of the pressure in the bladder at any time may be obtained. The position of the smaller tube is such that only when the sphincter is relaxed or opened will it be possible for fluid to escape by that path.

If now the sacral nerve supply to the bladder be stimulated it is found that the internal pressure of the bladder, as shown by the column in the vertical manometer, rises very considerably before there is any escape through the sphincter. If then, after the bladder has come to rest, and without stimulating in any way, the walls of the bladder be gently compressed between the thumb and forefinger, it is found that, when the pressure is raised to the same point, emission through the urethra takes place in exactly the same way. And again, the result is the same when the pressure is raised by admitting fluid slowly through a side branch inserted at the base of the manometer and communicating with a reservoir. Thus, for example, if it is found on mechanically raising the pressure, either through the T-tube or by compressing the bladder wall, that the sphincter is forced open only when the manometer shows 18 cm. (of normal

ing drugs, morphine, chloral hydrate, atropine, and cocaine, weaken the response of the detrusors and decrease the outflow through the urethra. Strychnine increases the detrusor reflex, giving at first an increased outflow through the urethra, a result to be explained as due to a slight increase in tone of both the centre for the detrusors and the tonic centre for the sphincter, the latter however not sufficient to over-balance the former. Later, strychnine gives an increased response on the part of the detrusors with *no* outflow through the sphincter, — doubtless due to such an increased tone of the sphincter centre that the centre is no longer inhibited by the usual stimulus. Muscarine and nicotine give, on injection, contraction of the detrusors and dilation of the sphincter without stimulation. I have obtained the same results with nicotine repeatedly, but have successfully avoided the contraction of the detrusor by injecting the nicotine slowly, at body temperature. The dilation of the sphincter results from an interruption of the physiological path of the tonic sphincter impulses, through poisoning of intermediate cell connections.

saline solution), it is invariably found that stimulation of the sacral nerves is ineffectual until the pressure has been raised to the same degree. And the result is the same whether the peripheral cut ends, or the nerves in their continuity be stimulated.

If relaxation of the sphincter may not be brought about by direct stimulation of its nerve supply, there still remains, however, a possible physiological method of producing relaxation. It has been shown that there is, at about the level of the V lumbar root, a centre in the spinal cord from which tonic impulses pass to the vesical sphincter.¹ The interruption of the connection with that centre has also been shown to produce incontinence of urine,² and to lead to a loss of power in the sphincter which reduces its resistance to nearly that which is present after death. If, too, the physiological connection be interrupted by the injection of nicotine the same result is obtained. Before an injection the sphincter, in one experiment, resisted successfully a pressure of 20 cm. of saline solution, but immediately afterwards yielded to a pressure of 4 or 5 cm., this residual power being due apparently to its elasticity.

It is possible by stimulating the central end of a sensory nerve to produce such a degree of interference with the tonic vesical centre that dilation of the sphincter will result. To demonstrate this the two catheters were inserted as before. In a typical experiment it was found that emission of fluid took place when the bladder pressure was raised to 22 cm. either through a T-tube or in response to stimulation of the uninterrupted sacral nerve supply. The bladder came to rest at a pressure of 9 cm. The central cut end of one sciatic nerve was then stimulated with a strong induced current, and although the pressure rose to 11 cm. for a short time no emission took place. The pressure was then raised through the T-tube to 18 cm., 4 cm. less than that normally necessary in this experiment to produce emission. The central end of the sciatic nerve was again stimulated, this time producing a rapid escape from the urethral cannula. In every case, though stimulation of the central end of the sciatic nerve would not of itself empty the resting bladder, it

¹ MASIUS: *Bulletins de l'académie royale de Belgique*, 1868, p. 491; GIANUZZI: *Della tonicità degli sfinteri dell'ano e della vescica urinaria*, 1869; reviewed in HENLE, MEISSNER, and GRENACHER's *Bericht über die Fortschritte der Anatomie und Physiologie*, 1869, p. 304; KUPRESSOW: *Archiv f. d. ges. Physiol.*, 1872, v, p. 291; OTT: *Journal of physiology*, 1879-80, ii, p. 41.

² GIANUZZI: *loc. cit.*

nevertheless relaxed the sphincter when the pressure was raised to a degree in itself just insufficient to overcome its tonic resistance, — a method parallel with that used by Langley in demonstrating relaxation of the cardiac sphincter.¹

Similar results were obtained on stimulating the central cut ends of both hypogastric nerves together. And, as already referred to, Courtade and Guyon have shown that stimulation of the central cut end of the sacral supply causes dilation of the sphincter, an experiment marred only by the fact that half the tonic impulses to the sphincter must necessarily be interrupted.

CONCLUSIONS.

1. The lumbar nerve supply to the bladder contains fibres, stimulation of which produces relaxation of the detrusor muscles, distinct from those which carry impulses causing contraction.
2. Stimulation of the sacral nerve supply of the bladder, whether of the peripheral cut ends, or of the nerves in their continuity, does not relax or inhibit the vesical sphincter.
3. Emission takes place under stimulation only when the normal tonic resistance of the sphincter is overcome.
4. Stimulation of the central cut ends of the lumbar supply, of the nerves of the sacral supply, or of the sciatic nerves, produces an interference with, or inhibition of, the tonic centre for the sphincter of the bladder, resulting in a relaxation or dilation of the sphincter.

¹ LANGLEY: *Journal of physiology*, 1898, xxiii, p. 407.

THE REACTION OF AMOEBA TO LIGHTS OF DIFFERENT COLORS.

By N. R. HARRINGTON AND EDWARD LEAMING.

[From the Departments of Pathology and Physiology of Columbia University at the College of Physicians and Surgeons, New York.]

A NUMBER of the lower plants are able to distinguish lights of different intensities. Others, such as the purple bacteria and swarm-spores, have been shown to respond in different ways to differently colored lights of presumably the same degree of intensity. The purpose of this paper is to show that the common *Amoeba proteus* possesses both these kinds of sensitiveness to light; its protoplasm may be set in motion or brought to rest by varying the light to which it is exposed. Further it will be shown that the response is quicker to some light rays than to others. Finally, certain observations on the structure and the contents of the cytoplasm and on the nature of streaming will be discussed.

Without claiming new methods of work, it may be pointed out that the use of the apparatus hereafter described and the employment of colored screens to promote streaming, enables us to approach from a favorable standpoint the difficult problem of the nature and meaning of protoplasmic flow. Light is easily controlled, and its action can be observed throughout the whole or in any part of the transparent organism.

The effect on streaming of lights of different colors. Method of work.—The amoebae,¹ which for a research of this kind must be abundant and large, were studied from the image projected on the

¹ The amoebae were procured for us from some decaying *Vitella* by the late Professor J. I. Peck, in Williamstown, Mass. While attempting to photograph one of the more active of these amoebae, it was found that by throwing bright white light upon the field, the animal could be held in any phase of pseudopodial formation long enough for the securing of a photograph. The rigor thus produced was immediately relieved by red, green or violet light. This discovery led to the investigation here reported.

The photography, arrangement, and manipulation of apparatus were entirely the work of Edward Leaming, and were done in his laboratory in the Department of Pathology, Columbia University. The remainder of the work was done by N. R. Harrington.

ground glass back of a large Zeiss photomicrographic apparatus by means of an electric arc lamp. The change in the color of the light was effected almost instantaneously by the interposition of the light-filters of colored celloidin used by Bierstadt in photographing colors. Although these screens do not give purely monochromatic light, there is always one predominant color in the field, and there is no reason to suppose that the very striking results produced are complicated much by a slight mixture of rays different from that of the principal color, such as the slight red transmitted by the violet. To satisfy any doubts in this respect, the experiments have been repeated, using Hartnack's illuminating apparatus for monochromatic light, and it has been found that the same results are obtained with monochromatic and orthochromatic lights. It was, however, impossible to determine latent periods with the former light because of the difficulty in changing instantly from one part of the spectrum to another. It would have been desirable to work with sunlight and monochromatic screens, were an apparatus procurable which would give a colored light as brilliant as that obtained by an arc lamp and colored screens.¹

A close comparison of the effect of pure and mixed colors shows that the slight impurities just discussed, for example, the faint red transmitted by the violet (to take the most striking case), are far less misleading than other factors which can never be entirely controlled. Such inaccessible factors are variability in moisture, oxygen, pressure, and capillary currents. It is necessary to take great care that temperature and intensity do not vary under the different color screens used.²

To show that variation in temperature was not a source of error, a delicate thermometer was suspended with the bulb in the position usually occupied by the amœba. After exposure for a considerable time to the hottest white light used by us, the mercury rose from 20.9°C., the temperature of the room, to 25.2°C. When celloidin films were interposed, the temperature settled to 24.8°C., and long exposures to red, green, and violet lights showed that no one of these films transmitted more heat than another. In order to make the temperature of the white uniform with that of the colored rays, a piece of mica was used with the former light.

¹ Since the above was written, the experiments described in the present article have been repeated with the spectrum, and results entirely confirmatory of those above described have been obtained.

² Jarring alone produced no perceptible constant effect.

It is very difficult to compare accurately the intensities of *transmitted* lights of different colors. The films might have been compared by Whitman's modification of the flicker method¹ had the process been known to the experimenters at the time. Screens were selected, however, that illuminated about equally an opaque screen and an observation was made which proved conclusively that the decisive factor is color, and not relative light or darkness. When an amœba is flowing rapidly, one violet screen tends to retard the flow. If now a second violet screen be inserted, still greater retardation is produced. The retardation cannot be due to the diminished intensity or brightness, for the normal effect of darkness after streaming is the resumption of streaming. The same can be proved in another way:—A yellow glass, which transmits rays of great intensity, will nevertheless allow the resumption of streaming after quiescence in violet. When the bright yellow is changed to a violet of much less brilliancy, streaming stops. Here is an instance of greater darkness and coincident retardation of flow. These facts show that in these cases color is a stronger factor than intensity.

Intensity, however, is a very important factor in phototonus as is shown by the active streaming which often takes place during darkness, as well as by the retarding effect of very bright light. The fact that two green screens produce greater flow than one might be accounted for by the lessened intensity or the effect of the color.

The following typical experiment illustrates the method:—

An amœba of spherical form was brought into place on the camera. It remained in the dark field a considerable time without perceptible movement. From darkness a red light was suddenly thrown on. In ten seconds, a movement was apparent in the inner cytoplasm and one crystal after another began to change its position. A current of these particles and of the ground-substance in which they were suspended finally became established, and a pseudopod of the entire width of the body was formed, flowing rapidly across the field and followed by the remaining parts of the body. The organism was kept in red for two minutes and the rapid flow was maintained. When the red film was replaced by a violet one, the streaming slowed instantly, and in five seconds there was a complete stop. Screens of different colors were successively employed. Whenever green or red films were inserted the movement started. It was checked by violet or white light. In two or three instances after a change from red to violet there was not only cessation of flow, but a reversal of the current to an exactly opposite direction. It hap-

¹ WHITMAN: Physical review, 1896, iii, p. 241.

pened once or twice that after quite long exposure a current became established under violet light. This could be stopped only by more violet or white light. Occasionally a movement started under mild white light and this was instantly checked by changing the position of the condenser so as to increase the intensity. Under the bright white light produced in this way, the amoeba usually assumed a tense rigor-like condition, but instead of a return to the spherical form, as might have been expected, the flowing was stopped instantly and that form of the body was retained which the amoeba showed when the brightest light was first flashed upon it. Even pseudopodia which were just starting or those which had attained considerable impetus, were often checked instantly. This light-rigor is doubtless identical with the light-rigor demonstrated by Engelmann¹ in bacteria and by Pringsheim² in *Nitella*. On the other hand, it was observed that when the organism was left under white light for some time, a very manifest spasmodic attempt to form pseudopodia occurred. Ordinarily this effort was not accomplished, it being strongly suggested that restraining and impelling forces were acting at the same time.

Experimental data. — The observations which we have made will be presented here in a condensed form. A few preliminary remarks and examples will make it easy to follow them.

In white light the amoeba usually appears tense, and as a rule no streaming of its protoplasm can be observed. When the light is changed to red, green, yellow, or even violet, streaming begins, after an interval or latent period. Sometimes the streaming is seen even in white light; it is then modified by the change to colored light. Similarly, the change from red to violet, or the reverse, increases or diminishes the protoplasmic flow. If the excitant be increased, by the use of a double color screen, the streaming may increase until the maximum is reached. The latent period differs with different colors. After white light, red will be followed by streaming sooner than will green. The time necessary for a given color to produce its characteristic effect is an index of the efficiency of the color in causing or retarding flow. It gives also the relative values of the colors with reference to their approach to the optimum color.

Mild white following bright white — streaming continues and is accelerated.

Violet following white : —

- (1) Flow started and continued for three minutes.
- (2) Pre-existing flow at first slowed, then started with a rush.

¹ ENGELMANN: Archiv f. d. ges. Physiol., 1879, xix, p. 1.

² PRINGSHEIM: Sitz.-Ber. der Akad. der Wissensch., Berlin, 1881, p. 504.

- (3) Flow started after fifteen seconds.
- (4) Pre-existing flow slightly lessened.
- (5) Flow started after one second.
- (6) Flow started after fifteen seconds.
- (7) Flow started and pseudopodia made in eight seconds.

Green following white: —

- (1) Flow started from pre-existing rest almost instantly.
- (2) Flow started from pre-existing rest almost instantly.
- (3) Flow started from pre-existing rest almost instantly.
- (4) Flow started from pre-existing rest after ten seconds.
- (5) Pre-existing flow stopped.
- (6) Slow pre-existing flow increased in thirty seconds.

Yellow following white: —

- (1) Flow started from pre-existing quiescence ; (repeated many times).
- (2) Pre-existing flow in one direction changed to flow in opposite direction.

Red following white: —

- (1) Diffuse flow started after few seconds.
- (2) Flow started instantly after quiescence ; (repeated many times).

White following violet: —

- (1) Momentary stop of pre-existing flow.
- (2) Instantaneous stop of pre-existing flow.
- (3) Increase of pre-existing flow.
- (4) Pre-existing flow stopped in thirty seconds.
- (5) Distinct slowing of pre-existing flow after ten seconds.
- (6) Increase of pre-existing flow after thirty seconds.

White following green: —

- (1) Pre-existing flow stopped ; (this was repeated many times and demonstrated to several observers).
- (2) Pre-existing flow in one direction stopped instantly and reversed.
- (3) After pre-existing quiescence flow started in two seconds ; (observed only once).

White following yellow: —

- (1) Pre-existing flow stopped almost instantly ; (observed several times).

White following red: —

- (1) Pre-existing flow stopped instantly ; (repeated many times).
 - (2) Pre-existing flow stopped in thirty seconds.
 - (3) Pre-existing flow stopped in three seconds.
 - (4) Pre-existing flow stopped in five seconds.
 - (5) Pre-existing flow stopped in three seconds.
 - (6) Pre-existing flow stopped in six seconds.
- } mild white.
} bright white.

Violet following green: —

- (1) Pre-existing flow stopped instantly; (observed several times).
- (2) Pre-existing flow checked and reversed.
- (3) Pre-existing flow reversed in fifty-six seconds.
- (4) Pre-existing flow stopped in twenty seconds.
- (5) Pre-existing flow stopped in twenty-five seconds.

Violet following yellow: —

- (1) Pre-existing flow stopped in twenty-four seconds.

Violet following red: —

- (1) Pre-existing flow stopped and reversed.
- (2) Pre-existing flow checked.
- (3) Pre-existing flow stopped in forty seconds.
- (4) Pre-existing flow stopped in forty seconds.
- (5) Pre-existing flow stopped in five seconds.

Deep blue following violet: —

- (1) Pre-existing flow slowed instantly.
- (2) Pre-existing flow slowed after short time.
- (3) Pre-existing flow stopped.

Green following violet: —

- (1) Internal flow at once started.
- (2) Flow started from pre-existing quiescence in fifty-six seconds.
- (3) Flow started from pre-existing quiescence in sixteen seconds.
- (4) Flow started almost instantly; (repeated many times).

Yellow following violet: —

- (1) No effect after pre-existing quiescence.
- (2) Flow started after ten seconds.
- (3) Instant increase of pre-existing flow.
- (4) Increase of pre-existing flow in one second.

Red following violet: —

- (1) Pre-existing flow in violet reversed in two seconds.
- (2) Flow started in three seconds.
- (3) Flow started in three seconds.
- (4) Flow started instantaneously.

Purple (yellow and violet) following violet: —

Pre-existing flow increased.

Violet following purple (yellow and violet): —

Pre-existing flow stopped in three seconds.

Green, red, and yellow lights have so nearly similar effects on the organism that there is generally no perceptible change in streaming

when these three colors are interchanged. Nevertheless, that red is the most powerful excitant to flow is indicated by the shorter latent period after quiescence in white light.

Some of the recorded actions under two colors, both from the red end of the spectrum, are:—

Green following yellow:—

- (1) Pre-existing flow stopped after twenty-four seconds and started in opposite direction after twelve seconds more.
- (2) Pre-existing flow changed to diffuse flow in other directions.

Yellow following green:—

- (1) No perceptible effect.

Green following red:—

- (1) No perceptible change.
- (2) Pre-existing flow changed to another direction.
- (3) No perceptible change.
- (4) No perceptible change.
- (5) No perceptible change.

Red following green:—

- (1) Increase of pre-existing flow.
- (2) No perceptible change.
- (3) No perceptible change.

Yellow following red:—

- (1) No perceptible change.
- (2) No perceptible change.
- (3) Slight increase of pre-existing flow.

Red following yellow:—

- (1) No perceptible change.

These results can be conveniently summarized in the following table, which gives an average latent period for the effects of each individual color as an excitant and as a retarder.¹ The value of the average, it should be stated, is somewhat impaired by the difficulty of fixing the exact moment at which the effect begins to show itself or reaches its maximum. The numerals in the table indicate the interval in seconds between the application of the color and the production of its characteristic effect. The sign + indicates that the particular sequence of colors allows streaming to begin or, if already present, to increase; similarly, the sign — indicates that streaming is

¹ These terms are employed in this paper simply for the sake of brevity and convenience; the cause of protoplasmic flow is still an unsettled problem.

stopped or retarded in the given number of seconds. The asterisk shows that the change of color has no perceptible effect.

	White Preceding.	Violet Preceding.	Green Preceding.	Yellow Preceding.	Red Preceding.
White following . . *		-5 ?	-1	-1	-1
Violet following . . +5		*	-20	-24	-9
Green following . . +5		+12	*	?+12	*
Yellow following . . +1		+3	*	*	*
Red following . . +1		+2	*	*	*

The table shows that the effectiveness of the following kinds of light as inhibitors of protoplasmic flow diminishes in the order named: white, violet, red. No experiments have been made in which the amœba has been long exposed to any one light for the purpose of ascertaining what particular wave-lengths are favorable to protoplasmic streaming. All the data collected above refer to the effects produced by *suddenly changing* five particular screens. In this connection, it should be stated that after a few minutes, streaming will commence under any light, if the amœba be a fairly active individual. An interesting problem lies open to the observer regarding the relative times which elapse before an amœba becomes attuned to lights of different colors.

The latent periods for two colors as sequent and precedent do not correspond. For instance, when yellow follows violet, streaming will start in three seconds; but when violet follows yellow, there is an interval of twenty-four seconds before any effect is observed. The effect, therefore, of yellow following violet is not exactly opposite to that of violet following yellow.

Reaction of an enucleated fragment.—If the water supply becomes partially exhausted, the amœba is apt to rupture and to pour out a large number of crystals with the surrounding ground-substance and oftentimes the nucleus. If water were added after the nucleus was extruded, it was found in one or two cases that the enucleated part seemed to heal rapidly and showed a faint streaming. Under red light the streaming was materially increased, but under white or violet suddenly checked. This reaction was obtained several times and the enucleated fragment survived three or four hours.

Structure and contents of the cytoplasm.—The granules in amœba, which have been described by Leidy and others as crystalline in structure, are constantly undergoing a vibration which suggests Brownian movement. This appearance is seen best under a very

high power. Under the high power it also may be observed that the ground-substance of amœba apparently contains a great number of small bodies which appear like active bacteria. As described by Leidy, the crystals are of several forms, the most prevalent being either barrel-shaped or spherical.

In the use of the apparatus above described, several opportunities were offered for observations on the structure of this protozoan. Some of these points are illustrated by the accompanying photographs of living amœba, which give interesting and vivid representations of protoplasm under a 1.5 mm. oil immersion objective. Fig. 1, Plate I, shows a contractile vacuole surrounded by homogeneous ground-substance. The homogeneous outer layer, generally called the ectosarc (ectoplasm), can be distinguished from the inner portions of the amœba only by the absence of granules. That this distinction is a purely arbitrary one is indicated by the specimens sometimes observed in which the granules are all aggregated at one end, leaving an entire end of the amœba composed of a translucent, homogeneous mass. The transparent half of one amœba of this kind was observed to contain seven large water (or contractile) vacuoles.

Since the granules may be carried by internal currents into all parts of the ground-substance, there is clearly no fundamental difference between the "clear peripheral substance, the ectoplasm, and a central substance, the entoplasm, filled with coarse granules."¹ If it is necessary to use the terms ectoplasm and entoplasm, the latter must be defined as that part of the general ground-substance in which crystals and granules are present, but it should be remembered that neither they nor the outer ground-substance are constant in position, for, as has been stated by Ryder and others, the inner and outer parts of amœba constantly interchange.

Figure 2, Plate I, shows the crystalline bodies, the edge of the ectosarc, and the nucleus.

Nature of streaming.—Streaming does not invariably seem to originate with or be controlled by that part of the protoplasm which is near the circumference. On the contrary, we have observed it to begin always near the centre of a mass of particles midway from either edge. This point may or may not coincide with the centre of the amœba itself. When an excitant light falls upon an amœba which is at rest, the first movement observed is often the shifting in

¹ SEDGWICK and WILSON: *General Biology*.

position of one or a few granules. These are followed by others until a general current is established, the clear substance always preceding in the formation of the pseudopodia. When fully established, the streaming of the particles proceeds rapidly in the centre of the mass, but much less rapidly near the edges.

Although we have been able by means of the apparatus used in the prosecution of these experiments to study the formation of pseudopodia under the most favorable optical conditions possible, with an effective and easily controlled means of stimulation, we have not been able to contribute to the difficult question of the homology of protoplasmic streaming with muscle-contraction. No evidence has appeared which confirms Verworn's theory that the spherical form of amœba corresponds to the phase of full contraction in a muscle-fibre. The spherical form is especially characteristic of inert amœbæ, but colors which produce a cessation of streaming do not cause the assumption of the spherical form. It often occurs that in an environment which is not favorable to streaming (intense light or heat) the amœba assumes a spherical shape, but, if protoplasmic movement be checked by retardant light, the organism is most apt to stop in just that position in which it happens to be, and to remain so, indefinitely.

SUMMARY.

1. Amœba streams in the presence of red light.
2. Streaming is retarded, stopped, or reversed by rays from the violet end of the spectrum.
3. Further, the effectiveness of the following kinds of light as inhibitors of protoplasmic flow diminishes in the order named: white, violet, red.
4. Enucleated amœbæ stream in red light, and cease to stream in violet or white light.
5. No confirmation for the theory that the circular form of Amœba represents full contraction has been found. It still remains to be proved whether or not pseudopodia are produced or retracted by contractions — local or general — of the outer portion (ectoplasm).

METABOLISM IN THE SUBMAXILLARY GLAND DURING REST AND ACTIVITY.

By VANDELL HENDERSON.

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THE data at hand on the question whether coincidently with the elimination of their characteristic products, the salivary glands take up nutriment during secretion, offer an apparent contradiction. Histological changes and the decrease both in weight and percentage of solids in an active gland indicate, as Heidenhain expresses it, that "at definite times the cells absorb from the blood or lymph definite substances; these substances are at definite times transformed; and at definite times these transformed substances are eliminated by the cells."¹ In contrast to this view, are the experiments of Pawlow.² His investigation was performed upon dogs in which the chorda tympani and sympathetic nerve supplying the left submaxillary gland were severed. Secretion was excited in the right submaxillary by electrical stimulation of both sciatic nerves, for periods varying from one and a half to five hours. The saliva obtained, as well as the active and resting glands, were analyzed for nitrogen by the Kjeldahl method. Ten active glands from the right side yielded 1.872 grams of nitrogen, and the saliva secreted by them 0.416 gram—a total of 2.288 grams of nitrogen; while the ten resting glands from the left side gave 2.177 grams of nitrogen. Commenting upon these figures, Langley³ points out that they "are not such as we should expect from the microscopical appearance of the gland cells. . . . The stimulated glands had lost during secretion about $\frac{1}{4}$ of their nitrogen-holding substance;" the saliva secreted contained approximately $\frac{1}{4}$ of the nitrogen of the resting glands, "so that presumably the active glands had taken up during secretion about 0.1 gram of nitrogen," or $\frac{1}{4}$ of the total amount. Thus, with regard to the nitrogenous or proteid metabolism of the active gland, anabolic and katabolic processes are to a considerable extent coincident.

¹ HEIDENHAIN: Hermann's Handbuch der Physiologie, v, p. 58.

² PAWLOW: Centralblatt für Physiologie, 1888, ii, p. 137.

³ LANGLEY: Schaefer's Textbook of physiology, 1898, i, p. 488.

The method employed by Pawlow is perhaps open to slight criticism, in that the stimulated glands were all on the right side; and apparently he did not exclude the possibility of errors due to exceptional variations between the glands on the two sides. Pawlow did indeed find the nitrogen content of the normal right and left submaxillary glands from ten dogs to be remarkably concordant, — amounting to 1.978 and 1.979 grams of nitrogen, respectively.¹ On the other hand, in eleven dogs Bidder found the left submaxillary heavier than the right; and Heidenhain reported a similar observation in two cases.

The question involved is of importance for the theory of secretion. It seemed desirable, therefore, to renew investigation with special reference to the criticisms outlined. The experience of the writer indicates that although differences do occur to the extent of fifteen per cent in the weight of the glands on the two sides, yet the preponderance is not more often on the one side than the other. As a rule the normal right and left submaxillary glands agree closely in weight, dry solids, and nitrogen content, as the following typical analysis shows: —

TABLE I.
Large dog of fifteen kilos.

	Weight of gland.	Dry solids.	Nitrogen.
	grams	grams	grams
Right submaxillary	8.48	2.136	0.2448
Left submaxillary	8.24	2.125	0.2424

The method employed in the present investigation was as follows: Dogs of various weights were allowed to fast for twenty-four hours, in order to insure a resting condition of the glands. Water was given *ad libitum*. Anaesthesia was maintained (after the subcutaneous injection of a small dose of morphine) by continued administration through a tracheal cannula of a chloroform-ether mixture in just sufficient amount to insure the quiescence of the animal. Secretion was excited by electrical stimulation of the chordo-lingual nerve for periods varying from one and a half to seven hours, and the saliva

¹ PAWLOW: *loc. cit.*, p. 138.

was collected. The choice of the gland for stimulation was not confined to one side. In order to discover how far differences in the weight and composition of the glands can be assigned to merely vascular changes, the conditions of experiment were varied. Thus, in a few cases the chorda going to the resting gland was not disturbed; in the others, it was severed at the same time as the stimulated nerve. In two cases the animals were killed with chloroform, while a vigorous stimulation was maintained. In the others, the dogs were bled to death at periods which were varied from thirty seconds to fifteen minutes after the last stimulation. In all cases both glands were removed immediately after death, carefully separated from their capsules, and weighed. They were then comminuted, and treated with absolute alcohol for several hours; the alcohol was evaporated off; the tissue was dried at 110° for eight hours, and weighed. Finally, the saliva and the solids thus obtained were analyzed for nitrogen by the Kjeldahl method. As the results of the experiments failed to show any noticeable differences assignable to the variations in experimental condition, the differences between the stimulated and resting glands afford an indication and a measure of the changes within the secreting cells. Tables II and III give the results of nine experiments.

The figures show that during activity the submaxillary gland undergoes a marked loss of weight. This loss, calculated on the total weight of the nine active glands, as compared with the resting, amounts to three per cent. If Experiment III be excluded, it amounts to seven per cent; and if Experiments VIII and II be considered separately, the difference in the weight of the active and that of the resting glands reaches even ten and thirteen per cent, respectively. The tables exhibit also a diminution in the solids of the active glands which, calculated on the totals for eight experiments, is equal to six per cent of the solids from the resting glands. If Experiment III be excluded from the totals, the difference in content of solids between the active and resting glands reaches eleven per cent; and in Experiment VIII it amounts to twenty per cent. The figures in the first columns in Table III justify the conclusion that in the secreting gland the content of solids undergoes a relatively greater diminution than does the total weight of the gland. The conclusions thus far reached are therefore in accord with those of Heidenhain. On the other hand, the nitrogen found in the active glands is only 1.4 per cent less than in the resting glands. The nine active glands

TABLE II.
Results of Analyses of Active and Resting Submaxillary Glands.

Number of Experiment.	Weight of dog.	Gland stimulated.	Duration of stimulation.	Volume of saliva.	Nitrogen in saliva.	Weight of glands.		Total solids.		Nitrogen.	
						Active.	Resting.	Active.	Resting.	Active.	Resting.
	kilos		hours	c.c.	grams	grams					
I . . .	14	Left	3	17.0	0.0201	4.63	4.60	1.0436	1.1136	0.0994	0.1032
II . . .	30	"	2	32.0	0.0185	9.00	10.34	2.4232	2.5640	0.2940	0.2925
III . . .	14	"	1½	17.0	0.0075	12.21	10.78	2.8930	2.6712	0.3525	0.3112
IV . . .	15	"	4	46.0	0.0180	5.97	6.22	1.5263	1.5754	0.1740	0.1785
V . . .	12	"	1½	10.5	0.0120	3.99	3.90	0.1134	0.1038
VI . . .	10	"	7	30.0	0.0120	3.38	3.58	0.7970	0.9135	0.0912	0.1020
VII . . .	8	"	3	35.0	0.0140	2.97	3.32	0.7120	0.8790	0.0832	0.0972
VIII . . .	12	Right	6	100.0	0.0387	4.65	5.14	0.9905	1.2516	0.1212	0.1470
IX . . .	9	"	1½	8.0	0.0027	2.73	3.13	0.6470	0.7840	0.0738	0.0882
Totals				295.5	0.1435	49.53	51.01	11.0326	11.7523	1.4027	1.4236

TABLE III.

Expressing results of analyses in percentages.

Experiment.	Solids in glands.		Nitrogen in glands.		Nitrogen in total solids.	
	Active.	Resting.	Active.	Resting.	Active.	Resting.
	Per cent.					
I	22.5	24.2	2.14	2.24	9.52	9.26
II	26.9	24.8	3.27	2.82	12.13	11.40
III	23.7	24.8	2.88	2.88	12.18	11.63
IV.	25.5	25.3	2.91	2.87	11.40	11.33
V	2.84	2.66
VI.	23.5	25.5	2.69	2.54	11.44	11.16
VII	24.0	26.4	2.80	2.92	11.69	11.05
VIII	21.3	24.3	2.60	2.86	12.24	11.74
IX.	23.7	25.0	2.70	2.82	11.41	11.25
Average.	23.9	25.0	2.76	2.77	11.50	11.10

contained 1.40 grams of nitrogen, the resting 1.42 grams, and the saliva 0.14 gram. The averages for the middle columns in Table III show identical figures for the *percentage* of nitrogen in the active and resting glands, and the experiments taken separately exhibit nearly as close an agreement. These observations, like those of Pawlow, indicate that the secreting gland tends to replace its loss of proteid to a considerable degree during activity.

An explanation of the apparent discrepancy between the figures for the total solids and those for the nitrogen in the resting and active glands, seems to the writer to be found in the columns giving the percentage of nitrogen in the solids. In every experiment the solids from the active gland were relatively richer in nitrogen than those from the resting. This difference seems to justify the assumption that the active glands had become poorer in carbon, hydrogen, and perhaps oxygen. It suggests, also, that within the secreting cells there was a vigorous combustion of carbonaceous material, — carbo-

hydrate, fat, the carbon moiety of proteid, or some such "explosive" combination of oxygen with substances rich in carbon, as is held to be the source of heat and work in muscle. Indeed, our knowledge of secretion points to such a combustion as the source of the energy liberated within the gland. Although recent investigations¹ have not verified Ludwig's² observation that the saliva is warmer than the blood, it is generally held to be very probable that a liberation of heat occurs during secretion. The saliva contains a much larger amount of carbonic acid than does the blood.³ Physico-chemical considerations prove that the separation of a fluid like saliva, containing 0.4-0.6 per cent of salts, from a fluid like the serum of the blood, containing 0.7-0.8 per cent of inorganic matter, involves work. Ludwig showed that saliva is secreted at a pressure higher than that of the blood in the carotid; and O. F. F. Grünbaum⁴ has recently published observations on the two kinds of work performed by the secreting glands. The microscopical changes observed in the secreting cells do not seem to offer any contradiction to the view that a combustion occurs. Observations of Heidenhain⁵ tend to show, also, that stimulation of the cervical sympathetic and the resulting secretion of a saliva of small volume but large content of organic matter cause a loss of solids in the gland which is considerably less marked than that resulting from the abundant secretion excited by stimulation of the chorda tympani alone. Finally, the view that in the submaxillary gland proteid metabolism is more or less distinct from the processes of combustion and liberation of energy, accords both with the accepted theory of nutrition in the organism as a whole, and with the hypothesis of Heidenhain⁶ that the elimination of water and salts and the elimination of the organic constituents of saliva are controlled by different mechanisms. For, on the one hand, in the proteid metabolism of the cells, controlled by the trophic nerve fibres, anabolism occurs to a certain extent coincidentally with katabolism. The secreting gland tends to remain in nitrogenous equilibrium. On the other hand, the processes controlled

¹ BAYLISS and HILL: *Journal of physiology*, 1894, xvi, p. 351.

² LUDWIG: *Wiener medicinische Wochenschrift*, 1860, pp. 433, 449.

³ PFLÜGER: *Archiv f. d. ges. Physiol.*, 1868, i, p. 686.

⁴ GRÜNBAUM, O. F. F.: *Journal of physiology*, 1898, xxii, p. 385.

⁵ HEIDENHAIN: *Studien des physiologischen Instituts zu Breslau*, 1868, iv, p. 66.

⁶ HEIDENHAIN: *Hermann's Handbuch der Physiologie*, v, pp. 50-51.

by the secretory fibres are apparently performed at the expense of a combustion of carbonaceous material stored within the cells during rest, to become the source of heat and secretory work.

In conclusion, the writer desires to acknowledge his obligation to Professor Lafayette B. Mendel, both for the suggestion of the subject of this investigation, and for valuable criticism.

STUDIES IN THE CONTRACTION OF SMOOTH MUSCLE.¹

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THE preparation used in the experiments here reported is taken from the frog's stomach. Two cross cuts are made from two to eight millimetres apart, and the ring of muscle thus obtained is suspended in a moist chamber between two metal hooks, one of which is fixed, while the other is connected to a muscle lever.

The muscle lever was provided with a pen of the style used by Pflüger. The pen swings freely on a horizontal axis set in the end of the lever and at right angles to the direction of the lever. This arrangement permits the lever to be placed perpendicular to the drum, instead of being tangent to it, as the ordinary muscle lever must be. Thus, the distortion which results from the curvilinear motion of the pen of the ordinary lever is avoided. The effect of this distortion, as seen for instance in the curves in Bottazzi and Grünbaum's recent paper,² is to make the ascent too sloping, and the descent too steep. The true time relations of the different phases of contraction are more plainly represented by such curves as are shown in Figure 1 or Figure 4 than by those drawn with tangential levers.³

SPONTANEOUS CONTRACTIONS.

As the shape of the single contraction-curve has been pretty thoroughly analyzed by previous writers, notwithstanding the criticism just made, I shall confine myself to the relations that exist between successive contractions, and in particular to the great variability in their heights and intervals, and to the striking rhythm that often appears.

Heights and intervals of contraction. — Morgen⁴ found the spontaneous contractions of the ring of smooth muscle continued for about

¹ A report of some of the results of this investigation was made by Dr. H. P. Bowditch at the meeting of the British Association for the Advancement of Science, in Toronto, 1897.

² BOTTAZZI and GRÜNBAUM: *Journal of physiology*, 1899, xxiv, pp. 51-71.

³ For the history of the subject, see H. WINKLER: *Archiv f. d. ges. Physiol.*, 1891, lxxi, pp. 357-368.

⁴ MORGEN, B.: *Halle Untersuchungen*, 1890, p. 144.

20 minutes and not longer. Our preparations have however retained their activity much longer, often for 18-30 hours after being suspended. In Dr. Lovett's experiments,¹ the average duration was —

In strips from the cardiac portion	10½ hours.
“ “ “ middle “	10½ “
“ “ “ pyloric “	7¼ “

During this long period the size of the contractions and the interval between them are undergoing continual variations. Seldom do we find a series as regular as is usual in the heart, and such a series never lasts long. The whole period of activity may perhaps be divided into four stages, though with the understanding that they are not always all present, nor ever separable with absolute sharpness. There is a short initial stage of frequent and vigorous contractions, often accompanied by a gradual rise in tonus. These contractions, however, soon become feebler, and in the second stage almost disappear, while the tonus also rapidly falls. Next, a gradual increase in the size of the contractions ushers in a stage of vigorous activity, even more vigorous than the first stage. The tonus, however, does not rise again, but continues very slowly sinking. This third stage is often very protracted, but passes at length into the still longer stage of progressive fatigue, during which the tonus, already nearly exhausted, diminishes but little, while the contractions become smaller and smaller and finally disappear.

In a set of three rings from the same stomach, the lengths of the different stages were as follows: —

	STAGE			
	1	2	3	4
First ring	½ hr.	1 hr.	8 hr.	15 hr.
Second ring	5 min.	2 hr.	7 hr.	16 hr.
Third ring	20 min.	½ hr.	7 hr.	17 hr.

Besides these slow changes in the condition of the muscle, there is a difference in the force of nearly adjacent contractions. Seldom do two successive contractions register the same height, seldom are two successive intervals equal. Both vary without any apparent regularity or law. Inasmuch, however, as both vary simultaneously, it is easily suggested that one variable may be dependent on the other. Thus, if the variation in height or force be taken for

¹ BOWDITCH: Report of the British Association for the Advancement of Science, 1897. pp. 809, 810.

granted, it might easily be conjectured that the stronger the contraction, the longer the pause that must follow for recuperation. Or, conversely, if the variation in intervals be taken for granted, it might be supposed that the longer intervals, affording more complete recuperation, would be followed by the higher contractions. As between these two it is not hard to decide. On measuring the tracings, it is found that the first conjecture will not fit the facts at all, whereas the second fits them remarkably well. In other words, the stronger contractions are not as a rule followed by the longer pauses, but the longer pauses are very apt to be followed by the stronger contractions. Pick out the highest contractions in a series, and you will almost always find them preceded by especially long pauses (see Fig. 3). In attempting, however, to apply this rule to details, we must make a certain amount of allowance for slow changes in the

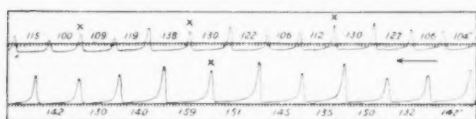


FIGURE 1. Spontaneous contractions. One third original size. Time in 10 second intervals. The numbers on the tracing give the time elapsing between the beginning of each contraction and the beginning of the next.

condition of the muscle. We cannot state rigidly that a pause of a given length is followed by a contraction of a given height. This is impossible even with the same piece of muscle, and within the limits of a single one of the four "stages" mentioned above. Within fifteen minutes, the condition of the muscle is sometimes so changed that the same length of pause is followed by contractions of quite different heights. But if we sufficiently confine our view, we find our rule that the longer pause is followed by the higher contraction to hold. It holds very well in the following modified form:—

"Of two *successive* contractions, that will be the higher which is preceded by the longer pause."

For a fair demonstration of this rule, see Fig. 1. The exceptions are here indicated by crosses.

The rule holds in more than 90 per cent of the individual contractions that I have examined. The exceptions can usually be explained by supposing that the contractility is more quickly restored to a certain level after a weak contraction than after a strong. We have stated that a high contraction does not have the effect of prolonging the following pause. But it may operate to make the next

contraction feebler than is usually the case after the given length of pause.

This supposition suffices to explain the exceptions in Fig. 1.

In other instances the operation of our rule seems to be obscured by the presence of a series of minute contractions, which mix in with the large. These must usually be counted out in applying the rule, and yet they interfere slightly with the size of the large contractions. One of them occurring just before a large contraction may make it smaller than the rule would require—and probably smaller than it would have been had the small contraction not interfered.

In the complex and irregular curves, therefore, our rule has many exceptions. But in the more regular series, it accounts very well for such variation as appears in the force of contraction. There is little room for doubt that the length of the pause strongly affects the force of the succeeding contraction. What the exceptions and complex cases show is that the length of the pause must be considered in connection with the recuperation to be accomplished in it, that is, in connection with the force of the preceding contraction.

In order to provide more satisfactory evidence in favor of this relation between the length of the contraction-interval and the force of contraction, I add here a table giving the measurements of a series of contractions which remained for a long time fairly regular. The length in seconds of the interval preceding any contraction is found in the column headed "Int.", and the height of that contraction is given next to it in the column headed "Ht." The contractions are recorded in order down one column and then without break down the next, and so on.¹ See Table I, page 30.

Among 95 cases, there are but 7 exceptions to the rule. And of these, the first three (*a*, *b*, *c*.) simply show equality of heights where the rule would call for a slight diminution, and two more (*f*, *g*.) show a change of not over 0.5 mm. in one direction, where the rule would call for a slight change in the opposite direction. The only striking exceptions are therefore *d* and *e*. Now *d* occurs at a

¹ The "interval" is reckoned from the beginning of each contraction to the beginning of the next. This interval is chosen because it can be more easily and more accurately measured than that from the beginning of relaxation to the beginning of the next contraction, and because the length of time occupied by the phase of shortening is nearly constant for all heights of contraction. "Pause" in the strictest sense there is none, since the muscle continues slowly relaxing up to the very beginning of the next contraction.

TABLE I.

Int.	Ht.	Int.	Ht.	Int.	Ht.	Int.	Ht.
51	42	181	44.5	122	42	154	37.5
27	27	28	26	89	36	217	40
110	43.5	154	43.5	111	39.5	119	32.5
26	27.5	26	24.5	96	37	122	36.5
153	43	111	39	88	36	195	39
31	28	137	41	100	42	148	35.5
174	45	105	39.5	85	35.5	129	32
27	27.5	197	45	81	34	201	40.5
132	43	26	27	131	41	174	38
120	43(<i>a</i>)	166	44	27	11	122	30
31	27	29	27	118	40	150	35
184	45.5	118	40.5	120	37.5(<i>d</i>)	127	29.5
28	28	144	43	85	31.5	156	37
159	44	28	25	107	34	239	39.5
30	28.5	132	41	100	33.8	153	31
186	45.5	121	41(<i>c</i>)	150	39	142	34.5(<i>e</i>)
25	29	30	19.5	157	41.5	235	38.5
140	43.5	153	43.5	127	36	234	35
30	27	113	40	146	38.5	161	31.5
99	38.5	131	42.5	91	32.5	203	35.5
104	40	144	43	75	31	193	35.6(<i>f</i>)
126	42	152	43.5	144	40.5	175	29.5
121	42(<i>b</i>)	32	28	141	37.5	190	36.5
31	27	91	36.5	110	31.5	215	36(<i>g</i>)

transition point, at which fatigue seems to make its appearance suddenly, since the contractions after it show a lower level, for the same length of interval than those before it. Finally, *e* (and *d* as well) can be explained by reference to the heights of the preceding

contractions, as suggested above. The exceptions seem therefore to have no weight as against the rule.

In Figure 2, the table is expressed in graphical form. Each case is denoted by a dot or cross or small circle, the ordinate of which represents the height of the contraction, and the abscissa the length of the preceding interval. There seem to be indicated in the table four stages of fatigue, the first extending to *d*, the second about half way from *d* to *e*, and the third to a little beyond *e*. The cases in the first stage are represented by the dots, those in the second stage by crosses, and those in the third stage by small circles. The fourth stage includes so few that I have omitted them from the plot. The dots are seen to lie very closely along a curve, the crosses along another, and the circles along a third,—thus indicating that, during each stage, the height of contraction is a definite function of the length of the preceding interval.

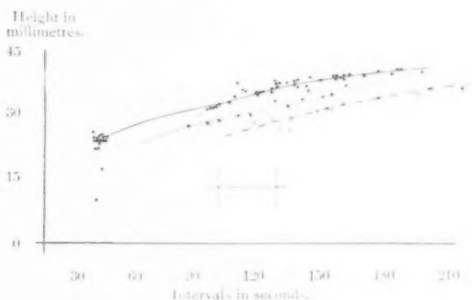


FIGURE 2.

As fatigue comes on, the function changes, the same interval now being followed by weaker contractions. But aside from such changes in the general capacity of the muscle, the height is determined, to within a narrow margin, by the preceding interval.

Comparison of smooth with cardiac muscle.—This "rule" will be of more interest when we consider it holds not only of the stomach muscle, but of the heart as well. The uniformity of the strength of the heart-beats is due, as I believe, to the uniformity in the intervals between them. And the irregularity in heights that is introduced by electrical stimulation is due to the irregularity in intervals so introduced. That is, the "extra contraction" is small because the interval preceding it has not allowed time for the accumulation of the regular amount of available energy or contractility. And the contraction following the compensatory pause is (often at least) greater than the regular beat, because the compensatory pause is longer than the regular pause. Langendorff¹ has indeed sought to show

¹ LANGENDORFF: *Archiv f. d. ges. Physiol.*, 1878, lxx, p. 481.

that the great height of this latter contraction results directly from the "abortive" character of the preceding extra contraction. He offers no evidence of a positive character except such as is equally in favor of the hypothesis here advanced. The simple expedient of introducing a second extra contraction at a varying interval after the first shows that the contraction following an "abortive" extra contraction is not of unusual height, except when the time intervening exceeds the regular interval between beats. The second extra contraction increases in size as the interval preceding it increases.

Thus the same rule is applicable to the heights of contraction of both stomach and heart muscles. It is applicable with even more rigor to the heart, because of the "all or none" law. Since each contraction of the heart, whether great or small, uses up all the energy at that moment directly available, the accumulation of a new supply must start, after each contraction, from zero. Very naturally therefore the amount available for the next contraction will be a function of the time elapsing before that contraction. In case of the stomach muscle, the available energy is not all consumed by each contraction, and hence the amount ready for the next contraction is not dependent entirely on the interval immediately preceding.

It must of course be understood that the above rule does not pretend to account for *all* variations in the strength of contractions, either of the stomach muscle or of the heart. It refers only to the differences between contractions that are near together. Either sort of muscle is subject also to more gradual changes in its capacity for work. In particular, the "staircase phenomenon" is evidence of an increase in capacity due not to rest, but to activity. The related fact, established also by Bowditch in 1871,¹ that the maximal height of contractions of the frog's heart is obtained, not by stimulating at the longest intervals, but at intervals of 4-5 seconds, is likewise an evidence of the beneficial effect of previous activity rather than rest. Both of these facts, and others of similar import, seem to militate against our rule. But (1) so far as facts go, there is no contradiction. The maximum interval obtained in my experiments between successive beats of the heart was less than 4 seconds, the optimum interval established by Bowditch. And (2) as concerns the interpretation suggested, there is again no contradiction. Both activity and rest are good for a muscle. If the pause preceding a contraction is too short, the contraction suffers from lack of recu-

¹ BOWDITCH: *Arbeiten aus der physiologischen Anstalt zu Leipzig*, 1871, p. 160.

peration. If it is too long, the contraction suffers from what we may perhaps call a sort of drowsiness. We find the same phenomena,—the optimal interval, the staircase, and the need nevertheless for intervals of rest,—in voluntary muscular action, and in various mental activities. In the case, however, of a series of the *spontaneous* contractions of heart or stomach, the intervals are comparatively small, considerably below the limits at which further rest ceases to be a benefit.

The fact referred to just above, that the "all or none" law does not hold good for the contractions of smooth muscle, has been observed in various preparations, by Fick,¹ Sertoli,² and recently by Barbéra.³ They found that the contraction increased with the stimulus.

A companion fact is that the spontaneous contractions, even when very strong, are seldom if ever maximal. A strong induction shock will produce a contraction greater apparently than even the strongest spontaneous contraction.

Nature of stimulus to spontaneous contraction.—From the above-mentioned facts we may legitimately draw certain conclusions regarding the internal stimuli that produce the spontaneous contractions. First, they must be of nearly uniform intensity for sometimes quite long series of contractions. For if they varied much in intensity, the contractions would vary with them. But we have apparently been able to explain the variation in height by the length of the preceding interval, leaving very little variation assignable to differences in stimulus. Another inference is that the normal stimulus cannot be either continuous or regularly periodic, but must occur at irregular intervals, corresponding to the observed intervals between the contractions. We are led to this conclusion by attempting to understand the irregular lengths of the intervals. We have already seen that the length of the interval is not explained by the height of the preceding contraction. And it will be clear that a contraction does not begin just when it does for the reason that the muscle is then just ready for it. If the occurrence of a contraction were dependent on a certain degree of recuperation in the muscle, the contractions ought to be of the same height irrespective of the length of the

¹ FICK: Beiträge zur vergleichenden Physiologie der irritablen Substanzen. 1863, p. 48.

² SERTOLI: Archives italiennes de biologie, 1883, iii, p. 89.

³ BARBÉRA: Zeitschrift für Biologie, 1898, xxxvi, p. 251.

pause. If we ask in case of a long interval why the following contraction did not come sooner, we cannot answer, Because the muscle was not prepared sooner to make a contraction. For on observing the great force of the belated contraction, we see that the usual



FIGURE 3. One third original size. Spontaneous contraction, at first with load, later with after-load. The record covers 3 hours.

amount of contractility must have been at hand some time sooner. We can only answer, Either there was no stimulus, or else the irritability was not restored as fast as the contractility. Or if we ask again, in case of a short interval, why the following contraction came so soon, we cannot reply that the contractility was restored sooner than usual. For the feebleness of the hastened contraction shows

that the contractility was not up to its usual level. We can only answer, Either the irritability was in this case restored very rapidly, more rapidly than the contractility, or else the muscle received, sooner than usual, a stimulus. In order therefore to explain the lengths of the intervals by reference to the irritability of the muscle, we have to assume that the curves of returning irritability and contractility, which in most muscles are very similar, have here no sort of parallelism. After one contraction the irritability must be assumed to be more rapidly restored than the contractility; after another, perhaps the next, this order is reversed. Unless we are prepared to make this violent assumption, we are driven to seek the cause of the irregularity of the intervals in the stimulus that causes the contractions. The internal stimulus must be supposed to be irregularly periodic.

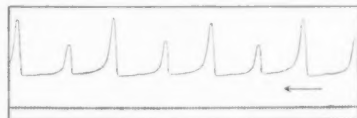


FIGURE 4. Spontaneous contractions. One third original size. Time in 10 second intervals.

As regards the character of the stimulus, my experiments have nothing positive to tell. They do serve, however, to exclude one plausible hypothesis, namely that the so-called "spontaneous" contractions are in reality nothing but responses to the mechanical stimulus of stretching by the weight of the lever. This hypothesis is excluded by the fact that after-loading the muscle (as in Fig. 3), or counter-weighting the lever, does not stop the contractions, nor necessarily diminish their height.

The irregularity of the contractions of the stomach muscle, though

often a hindrance to study, is in some ways likely to be a help. Variations which in the heart muscle are very slight are here paralleled by variations so pronounced as to be easily studied. Perhaps the most interesting of these variations are those which are more or less periodic, and which may be classed under the head of rhythm.

Rhythm.—The simplest rhythmical form—after the fairly regular recurrence of nearly equal contractions—is the repeated alternation of large and small contractions. Of this type there

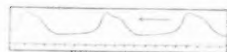


FIGURE 6. Spontaneous contractions. One third original size. Time in periods of 10 seconds.

are several varieties. In Fig. 4, the large and the small contractions are entirely separate, and the relation of height to interval follows the rule developed above. This form has already been described by Schultz.¹ Notice that the curve in Figure 4 is complicated by the presence of very minute contractions, but that these are strictly rhythmical in their recurrence. In the lower line of Fig. 5, the smaller contraction is superimposed on the larger. So also in Fig. 6, with the difference that the smaller contraction precedes the larger—a less common form.

Similar to the group of two is the group of three, the first the strongest, the last the weakest. This, though uncommon, is a very distinct type, and is sometimes repeated for hours at a time.

A less rhythmical interweaving of large and small contractions is seen in Fig. 7.

In Fig. 8 we seem to see a rhythmical type in the processes of formation, and later of going to pieces.

And the lower line of Fig. 5 shows, what is not uncommon, the reorganization of a rhythmical type after it has once been broken up.

Still more suggestive are instances of compound rhythm, showing a remarkable recurrence of the same group of contractions, with the same heights, the same intervals, and even the same slopes of ascent and descent. By the kindness of Professor Bowditch, I am enabled



FIGURE 5. Spontaneous contractions of two strips from the same stomach. One third original size. Record covers 17 minutes.

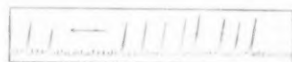


FIGURE 7. Spontaneous contractions. One third original size. Record covers 15 minutes.

¹ SCHULTZ: *Archiv für Physiologie*, 1897, p. 324.

to present here Figs. 9 and 10, which were shown by him at the meeting of the British Association in Toronto.

In the upper line of Fig. 9 we see a group of three contractions repeated, in the lower line a still more complex group of five. Yet more interesting is the middle line of Fig. 10. In looking at this tracing it is impossible, as Professor Bowditch remarked, to avoid the impression of an *interference* between two sets of contractions of different wave lengths, sometimes reinforcing each other, and sometimes acting separately. This tracing corresponds to the interference



FIGURE 8. Spontaneous contractions. One third original size. Record covers $4\frac{1}{2}$ hours.

of two independent sets of contractions, one acting with intervals of 108 seconds, the other with intervals of 97 seconds.

A ready interpretation of these results — as yet however a purely hypothetical interpretation — is that the different sets of contractions are the contractions of different sets of muscle fibres. This conception is almost forced upon us also in examining such tracings as are given in Figs. 5, 7, and 9. Sometimes, we may suppose, the different sets of fibres pull together and produce a few strong contractions; again they act separately and produce more numerous but smaller contractions. Sometimes they act in the same rhythm; again their period is different, and the result is the phenomenon of interference.

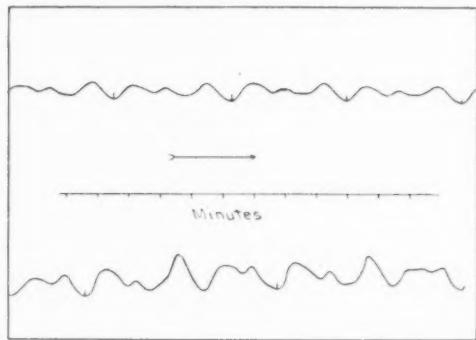


FIGURE 9. Spontaneous contractions of two strips from the same stomach.

Effect of heat on spontaneous contractions. — Grünhagen¹ and Morgen² have observed that heat always reduces the tonus. Morgen

¹ GRÜNHAGEN'S Lehrbuch, 1886, ii, pp. 121, 122.

² MORGEN: Halle Untersuchungen, 1890, p. 165.

found that cooling increased the tonus. It is not true, however, that the tonus which is lost by heating from 15° C. to 30° is restored on cooling again to 15°. The tonus is lost permanently. Schultz¹ found that the effect of heat on the spontaneous contractions was to increase their sharpness. I would add to this, that heating, if not too sudden nor carried much above 25-30° increases the number and reduces the size of the contractions.² See Fig. 11.

If the rise in temperature is more rapid or extreme, the contractions disappear altogether. But unless the temperature has been carried above about 40°, they reappear and gradually regain their strength. What is more surprising is that they will do this even if the temperature is maintained at the point which at first caused them to grow weak or disappear. See Fig. 12. This would suggest that the cause

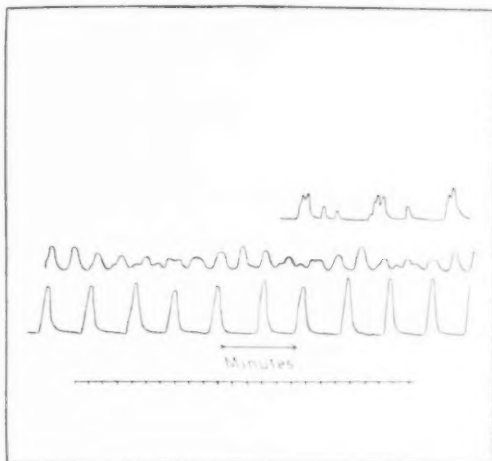


FIGURE 10. Spontaneous contractions of three strips from the same stomach.

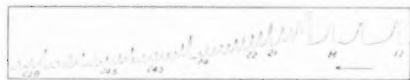


FIGURE 11. Spontaneous contractions affected by heating. One third original size. The numbers give the temperatures. At II, heat was first applied. Record covers 25 minutes.

of their disappearance was not the high temperature in itself, but the *sudden change* from a lower to a higher temperature. And it is

¹ SCHULTZ: *Archiv für Physiologie*, 1897, p. 325.

² In the interesting paper, just published by Bottazzi and Grünbaum on plain muscle, in which the œsophagus of toads was employed, similar effects of heat were noted and illustrated by a similar tracing (*Journal of physiology*, 1899, xxiv, p. 63, and Fig. 13). There are two differences, however, between the two results. The œsophagus shows no effect of heat below 29° C., while the stomach muscle shows a decided effect at 20° C. And although the œsophagus shows a decrease of tonus above 30°, it shows an increase from 29° to 39°. In the stomach muscle, I have never seen an increase of tonus in response to heat, and, indeed, have never failed to find a decrease in response to even a slight increase in temperature.

reasonable to suppose that the frog's stomach muscle, like the frog himself, though temporarily affected by sudden thermal changes, has considerable power of adaptation to various temperatures.

CONTRACTIONS FROM ARTIFICIAL STIMULATION.

Induction shocks.—The shape of the curve obtained by strong shocks differs from the curve of a spontaneous contraction in that it

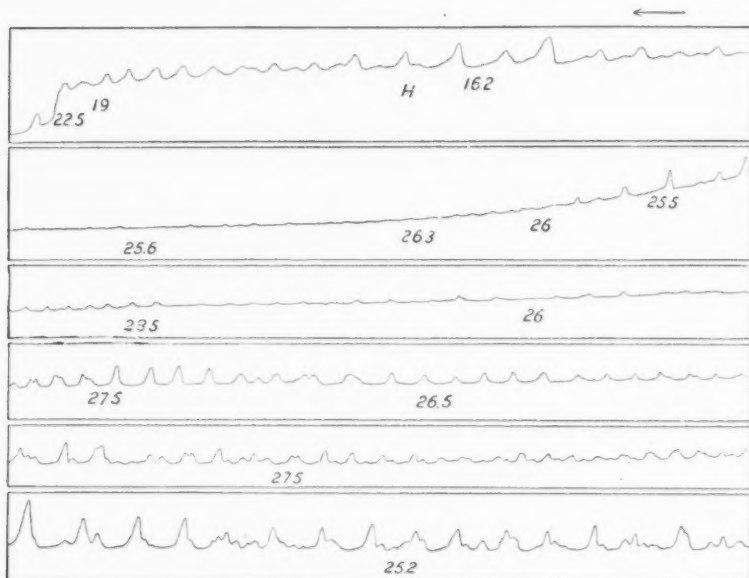


FIGURE 12. Spontaneous contractions affected by heating. Record is continuous, and covers 1 hour, 20 minutes. Numbers give temperatures. Heat first applied at H.

is simple, never presenting the double or triple character that so often appears in the curve of spontaneous contractions. It also tends to rise more rapidly and sink more slowly. See Fig. 13. The slow descent, though sometimes very marked, is so often slight or imperceptible as to make Winkler's emphasis of it appear an exaggeration.¹

It is of interest to compare the response of the stomach to an induction shock with that of the heart, especially as such terms as *refractory period* have lately been used in speaking of the stomach.

¹ WINKLER, N.: Archiv f. d. ges. Physiol., 1891, lxxi, p. 380.

We have already seen that the "all or none" law does not hold of the stomach. And it is for this reason, no doubt, that a true, absolute, systolic refractory period does not appear in the stomach muscle. Stimulus at any phase of the contraction will, if strong enough, call out an "extra contraction."

We do find, however, as the result of a forced contraction (as in Fig. 13), or sometimes though seldom as the result of a very powerful spontaneous contraction, a marked diminution of irritability and contractility. This is more in evidence during the descent than during the ascent of the curve. See Fig. 14. The diastole, rather than the systole, would accordingly be the refractory period. The heart muscle shows a similar low, gradually rising irritability during relaxation, — a phenomenon not to be confounded with the true refractory period.

A series of forced contractions ends by removing from the muscle all power of response to stimulus. Ten, fifteen, or twenty minutes must often elapse before any perceptible contraction can be got, and the full restoration of the contractility may take an hour or more. But I have never found a permanent destruction of irritability as a result of forced contractions, and suspect that when

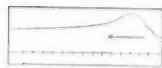


FIGURE 14. One third original size. Strong induction shocks at intervals of 10 seconds.

Winkler announces such a result,¹ it is because he allowed the muscle insufficient time for recovery. But I can entirely agree with him that the induction shock has a much worse effect on the muscle than the constant current. After a strong spontaneous contraction, or after a contraction in response to the make or break of the constant current, the condition of the muscle is at first not nearly so bad as after a contraction in response to a strong induction shock. The low irritability and contractility are in the latter case due less to the contraction itself than to some internal effect of the induction shock.

The *compensatory pause* also does not appear in the contraction of stomach muscle. Yet it too is stimulated to a degree. If a spontaneously contracting muscle is stimulated and gives an extra contraction, the spontaneous contractions generally cease. After a pause,



FIGURE 13. One spontaneous and two forced contractions. One third original size. The stimuli were strong induction shocks, make and break close together. Record covers 2½ hours.

¹ WINKLER: Archiv f. d. ges. Physiol., 1891, lxxi, p. 380.

or interval of quiet—which however has none of the exactness of the true compensatory pause and does not compensate—they again appear, but at first are almost imperceptible. They gradually increase until they equal or surpass their former size. This interesting variety of the "staircase" phenomenon is illustrated in Fig. 15.

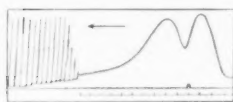


FIGURE 15. One third original size. A spontaneous contraction, and a contraction in response to an induction shock. Then, a "staircase" of spontaneous contractions. Time in 10 second intervals. During the staircase, the drum remained at rest, being simply moved along 2 or 3 millimetres after each contraction.

By repeated induction shocks a state of continued contraction is obtained. This *tetanus* (if it can be called such) differs from that of striped muscle in that it does not stay at its maximum height, but immediately begins to descend. Sometimes we get no better tetanus than that shown in Fig. 14. And I never have got, with induction shocks, so good a specimen as that in Fig. 16, which was obtained by use of the constant current.

The lack of a good plateau is easily explained by the low contractility after a forced contraction. The reason why a better plateau is obtained by the use of the constant

current is that the latter does not exhaust the muscle so much as the strong induction shock that is generally necessary in order to get any result.

In order to produce a smooth curve as distinguished from a series of scallops, the interval between successive shocks need not be small. Sometimes fifteen seconds is small enough. But increasing the frequency may increase the height of the tetanus.

I have tried to determine the smallest interval at which two shocks produce a different effect from one. It is difficult if not impossible to make an exact determination. Internal changes in the condition of the muscle are practically sure to vitiate any such comparison. What I have therefore done is to determine, in various specimens,

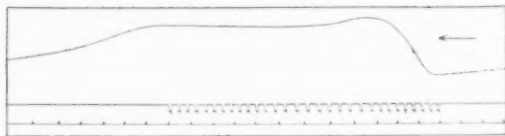


FIGURE 16. One third original size. Tetanus by repeated makes and breaks of a constant current (6 Daniell cells) passing through the muscle. In the line recording the stimuli, *make* is denoted by a fall, *break* by a rise. Time in 10 second intervals.

the smallest interval at which two stimuli can come in order that the effect of each may show separately on the curve. This interval varies widely in different specimens. Sometimes 2 seconds is sufficient, sometimes 10 seconds is too short. In most cases, the limit is somewhere about 7 seconds.

Constant current.—

Often either the make or the break of a current of 3-6 volts produces a contraction. The break contraction is usually the stronger.

If, however, the make is immediately followed by a break, or the break by a make, no effect is produced. There must be a perceptible interval in order

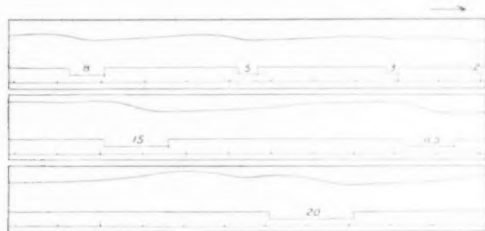


FIGURE 17. One third original size. Constant current, 3 volts. Make followed at a varying interval by break. Down = make, up = break. Continuous record. Time in 10 second intervals.

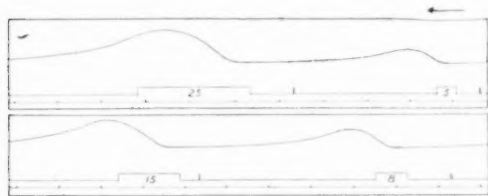


FIGURE 18. One third original size. Constant current, 3 volts. Break followed at a varying interval by make. Down = make, up = break. Continuous record, time in 10 second intervals.

to get any response. And as the interval is increased, the response increases. See Figs. 17, 18.

There is no summation of make and break, for the response to make is at its maximum when the current is allowed to flow with no break for per-

haps 20-30 seconds.¹ This will seem an argument for the view of Biedermann,² which is perhaps the received opinion, namely, that not only the make and break, but also the passage of the current acts as a stimulus. But that this is not the true conception appears in the complementary fact that a break (as in Fig. 18), immediately followed by a make, produces no effect, whereas if an

¹ These facts, though not entirely new, have not, I believe, been described for the muscle here in question. See FICK: *Beiträge zur vergleichenden Physiologie der irritablen Substanzen*, 1863, p. 48.

² BIEDERMANN: *Electrophysiologie*, p. 157.

interval be left, *during which no current is flowing*, the response occurs, and more strongly in proportion to the length of the vacant interval. If then we see in one case an exciting effect of the mere passage of the current, we are logically driven to see in the other an exciting effect of the mere absence of a current. Moreover such a record as that in Fig. 18 seems a sufficient disproof of any stimulating effect of the mere passage of the current. On this record the current is seen to have been passing through the muscle nearly the whole time. But no effect is visible except when the current is stopped.

The reason why the interval between make and break or between break and make is necessary is, I believe, that the two are antagonistic.

Make often produces not a contraction but relaxation from existing contraction. See Fig. 19.

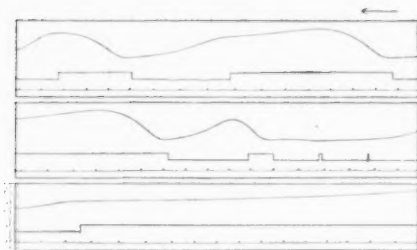


FIGURE 19. One third original size. Constant current, 3 volts. Antagonistic effects of make and break. In the line recording the stimuli, make is denoted by a fall, break by a rise. Continuous record, time in 10 second intervals.

Sometimes, indeed, make produces a contraction. But at the electrode from which the excitation starts, the processes set up by make and by break are probably always antagonistic in character. Why the total effects on the muscle should be now in the same direction, now in opposite, I do not attempt to say.

That fact remains. But if we admit that the *primary* effects of make and break on the muscle are always antagonistic, we can readily see why in all cases a perceptible interval is necessary between the two in order to get any contraction. The full development of the excitation due to either make or break takes, in accordance with the well-known character of smooth muscle, considerable time. This development can therefore be checked at any time within several seconds by the coming of the antagonistic process. In fact, examination of the three contractions in the lower line of Fig. 19 shows clearly that the reason why the longer interval gives rise to the stronger contraction is that it allows more time before the make comes with its relaxing influence. And in general, what makes the interval important is not the passage of the current, but the mere lapse of time, affording opportunity for the full development of the excitation.

The effect of a series of makes and breaks is in Fig. 20 seen to be sometimes contraction, and sometimes relaxation. It is contraction when the series begins with a break, relaxation when it begins with a make. Or, in the bottom line, it is contraction when the breaks are followed by longer intervals than the makes, and relaxation when the makes are followed by longer intervals than the breaks. But whether the effect be relaxation or contraction, it is not so strong as the effect of a single make or break.

These results may be readily interpreted in accordance with the principle that make and break are antagonistic. We know that the contraction wave in smooth muscle has a very slow rate of progress along the muscle, and a very great wavelength. That means that from the moment when the phase of contraction reaches a given cell till the moment when the phase of relaxation reaches it, a considerable interval must intervene during which the cell remains

contracted. Throughout the whole length of the muscle, the cells must be contracted at the same time. But let an artificial phase of relaxation—started by a make shock—follow close behind the phase of contraction. The result is that each cell remains in contraction for much less than its usual time, that few cells are contracted at the same instant, and that consequently the muscle as a whole does not perceptibly shorten. If, however, alternate phases of contraction and relaxation, produced by a rapid series of makes and breaks, run successively along the muscle, the result is that several short rows of cells are in contraction together, and the whole muscle is shortened to a degree. Or, in case the muscle is contracted to start with, the effect of a series of makes and breaks will be to bring into relaxation several short rows of cells that would otherwise have remained contracted, and so to produce a certain amount of lengthening on the part of the muscle as a whole.

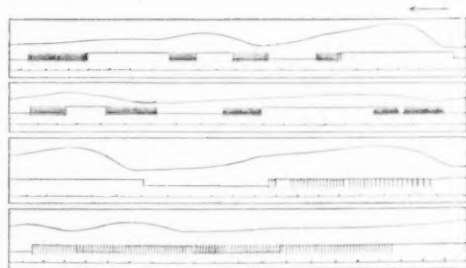


FIGURE 20. One third original size. Constant current, 4 volts. Effect of a series of makes and breaks. Down = make, up = break. Record of upper two lines continuous, and of lower two lines continuous.

Whatever may be thought of these details, the tracings in Fig. 20 show at least the antagonism of make and break, and the importance of this antagonism, as compared with the mere passage of the current, in determining the degree of contraction or relaxation of the muscle.

SUMMARY.

The frog's stomach muscle will often keep up spontaneous contractions for 18-30 hours.

The contractions are exceedingly variable in every respect, in strength, in shape of curve, and in the length of the intervening intervals.

The variability in strength is largely the result of the variability in the intervals. The longer the interval preceding a contraction, the stronger in general is the contraction.

The tracings show various sorts of compound rhythm.

Heating reduces the tonus, and diminishes the size but increases the frequency of the spontaneous contractions.

It is the sudden rise in temperature, rather than the high temperature itself, which enfeebles the contractions.

The smooth muscle has no "all or none" law, and its spontaneous contractions are not maximal.

It has no true refractory period or compensatory pause. But the irritability and contractility are much reduced by a forced contraction, and return but slowly.

Hence the tetanus of smooth muscle is quickly exhausted.

The make and break of the constant current have antagonistic effects on the smooth muscle, and neutralize each other unless a sufficient interval elapses between them.

The mere passage of the constant current through the muscle has no exciting effect.

A COMPARATIVE STUDY OF REFLEX ACTION AFTER
COMPLETE SECTION OF THE SPINAL CORD IN
THE CERVICAL OR UPPER DORSAL REGION.

By BENJAMIN MOORE AND HORST OERTEL.

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FEW of the fundamental physiological laws have been so well established and generally acknowledged as the theory of reflex actions and the relation of the brain to them.

"The spinal cord is the reflex centre, reflex action takes place as long as the arc is intact, the brain has an inhibitory power upon the reflexes and if this be removed the reflexes must necessarily be exaggerated."

This doctrine has impressed the minds of physiologists and pathologists to such an extent that not until recent years have cases been reported by Bastian and others which evidently contradict it.

The work on this question has hitherto been done almost wholly by clinicians. The physiologist has taken little part in it, and experimental study has been lacking until quite recently save for the observations of two authors, Rosenthal and Mendelssohn, and even their work seems to have been forgotten by more recent writers.

Long before Bastian reported his cases Rosenthal and Mendelssohn¹ published certain experimental observations on reflex action which seemed to them to support the view that a reflex might either take place at or near the level at which the afferent impulse reached the cord, or that the afferent impulse might first travel up to the medulla and there originate an efferent impulse. They further supposed that the longer path was the easier of the two and therefore that which was normally taken when the connection between cord and medulla remained intact; but when this longer path was severed or the stimulus increased beyond a certain limit they suggested that the shorter path was the one taken by the impulse.

¹ ROSENTHAL: Sitz.-Ber. d. kgl.-preuss. Akad. d. Wissensch., Berlin, 1873, p. 104; Biologisches Centralblatt, iv, p. 247; MENDELSSOHN: Sitz.-Ber. d. kgl.-preuss. Akad. d. Wissensch., Berlin, 1882, 1883, 1885. See also ROSENTHAL and MENDELSSOHN: Neurologisches Centralblatt, 1897, xvi, p. 978.

The experiments quoted in support of these views were made upon the frog and are briefly as follows: 1. The reflex time is shortened as the strength of the afferent impulse is increased. 2. The strength of stimulation necessary to provoke a reflex is increased when the spinal cord is separated from the medulla. The region concerned in this activity was found to lie at the apex of the *calamus scriptorius*. If the cord were cut at this point the strength of stimulus had to be considerably increased after the section in order to produce a reflex as before.

The experimental evidence in favor of this new view that spinal reflexes are normally discharged through the medulla seems to us to be insufficient; in the first place, it is natural to suppose, even if the path taken is the same, that stronger impulses should traverse it somewhat more rapidly; and again the difference in time experimentally observed is too short for the delays that would occur on account of the larger number of nerve cells interposed in the reflex arc. With regard to the second point, namely, the depression of reflex excitability after complete section of the cord, it is possible that these results may have been due to inhibition from irritation of the cut end (as in Türck's experiment), for in our experiments on the frog detailed later on we have found diametrically opposite results.

Bastian's report based upon observation of clinical material appeared in 1890.¹ He observed that in complete section of the cord high up in man, the reflexes below the cut, with perfectly intact arc, are not only not increased but are entirely abolished, even after secondary degeneration has taken place.

Bastian's results have been corroborated by further clinical and anatomical examinations, and we may refer for a detailed study of this now well-established clinical side of the question to the article of Bruns,² and the more recent publication of Senator.³

But while Bastian was inclined to think that in a complete destruction the reflexes were always absent we now know that such is not the case, for Senator has described three cases which were exceptions to this rule, and in one of these the reflexes remained exaggerated until the death of the patient, although the substance of the cord had been completely destroyed by a sar-

¹ BASTIAN: British medical journal, 1890, p. 480.

² BRUNS: Archiv für Psychiatrie, 1893, i, xv, p. 759; 1895, xxviii, p. 97.

³ SENATOR: Zeitschrift für klinische Medicin, 1898, i Heft.

comatous growth as shown by subsequent histological examination.¹

All these diverging clinical data have finally led to fresh experimental investigation of the subject and during the time we have been engaged in the present research two articles have appeared bearing upon the question by other writers.

Brauer² in a short paper reported an observation on a monkey. Immediately after section the reflexes were absent, but later, they gradually returned and finally were about normal. In view of this fact and the exceptions to Bastian's rule, Brauer does not consider any new formulation of the law of reflex action to be required. He states that the nearer the section to the reflex are the more exaggerated are the reflexes, and the further away, the more they are abolished, and he thinks that unknown factors may cause these abnormal effects, which are in his opinion insufficient to upset the well-established theory of reflex action.

The most recent contribution to the subject is contained in an extensive article by Sherrington.³ This author finds that division of the spinal cord in monkeys above the first cervical segment causes at first profound shock, but that after twenty minutes the skin reflexes begin to reappear. The knee jerk, however, may be lacking for days and weeks. Reflexes were also obtained by stimulating the nerve roots after total section of the cord. In addition to these effects permanent inhibitory phenomena were observed depending upon what the author describes as "isolation alteration." The view is here expressed that in man such results may remain sufficiently permanent to prevent the recurrence of any reflexes. It is also pointed out in this paper that a marked difference exists between monkeys and dogs, the latter resembling frogs by showing increased reflex action after section of the cord while the former show in this as in other things a close relationship to man.

From the preceding account it is evident that in the explanation of these phenomena two sides have been taken by the various observers, which may be respectively termed the anatomical and the functional view. Some, like Bastian, Rosenthal and Mendelssohn, suppose

¹ The destruction of the cord took place very slowly in this case, and the paralyzed condition and accompanying exaggerated reflexes persisted for a lengthened period previous to the death of the patient.

² BRAUER: *Münchener medicinische Wochenschrift*, 1899.

³ SHERRINGTON: *Philosophical transactions*, London, 1898, p. 136.

that reflex impulses must travel, with certain still obscure exceptions, through the upper part of the cord, and that transverse section destroys the normal path. Others, and to this class belong most clinicians, believe that section may produce functional disturbances which travel down the cord and in this way inhibit the normal change of sensory to motor impulse.

Our own work was undertaken with the special idea of observing reflex action after complete section at a high level in different animals beginning with the frog and ending with the monkey, and is here submitted chiefly as a study of the comparative physiology of the subject.

Experiments on frogs. — Twelve animals in all were operated upon; the cord was cut completely at the level of the first or second vertebra, and reflexes were produced either by application of acid, by pinching or touching, or by electrical excitation of the skin by the induced current.

The animals were carefully observed from day to day, and it was found in all cases that the reflexes were greatly increased above the normal. In many cases a slight pinch was sufficient to provoke a series of clonic spasms. It was noticed, however, that the limbs after section of the cord became fatigued exceedingly rapidly, so that the reflexes although at first exaggerated on repeated stimulation became feebler and in the end almost disappeared. After a prolonged rest it was found that the exaggerated reflexes once more returned.

The rabbit. — The cord of a large rabbit was completely severed at the level of the ninth dorsal vertebra; immediately after the operation there was paralysis of the hind legs and the skin and tendon reflexes were exaggerated. This condition persisted for a period of about twenty-four hours, at the end of which the animal died.

The cat. — 1. The cord of a large anaesthetized cat was exposed and a length of about one centimetre removed in the dorsal region. Five hours after the operation the tendon reflexes were found to be excessively exaggerated in the hind limbs, which were completely paralysed.

The reflexes were examined daily in this animal for a period of twenty-three days, and remained excessive throughout the whole of this time. The posterior portion of the animal became exceedingly emaciated towards the end of the experiment, and it continuously lost in weight although supplied with a liberal meat diet. The leg muscles remained in a soft flaccid condition and gradually became atrophied, although, as stated above, the reflexes continued to be exaggerated.

Towards the end of the experiment, the animal commenced to gnaw its hind legs (showing the complete absence of sensation) and a considerable loss of blood was sustained in this way.

The animal died at the end of the period mentioned above and a post-mortem examination showed that death was caused by intestinal obstruction which had formed in spite of all our efforts to prevent it by daily enemata, etc. On the two days succeeding the operation the animal's urine was removed by the catheter, but subsequently it was voided reflexly. The autopsy showed that the section of the cord had taken place at the level of the ninth dorsal vertebra.

2. A second cat had its cord tied very firmly with a silk ligature in the dorsal region, and made a rapid recovery from the operation. Five hours afterwards the reflexes were exaggerated and continued so during the entire period of observation, which lasted for twenty-six days, after which the animal was killed by chloroform.

The appearances presented in this animal were exactly those observed in the first experiment, but the muscular atrophy was even more pronounced, and probably for this reason the reflexes became somewhat lessened in the last few days.

3. In a third cat, the cord was completely severed in the cervical region. Immediately after the operation the breathing became diaphragmatic. An hour later the patellar and skin reflexes were obtained in a somewhat exaggerated degree. The muscles of the hind limbs remained stiff, due to a state of tonic contraction. About two hours later similar results were obtained. The animal died during the night and an autopsy showed complete section of the cord at about the level of the fifth cervical vertebra.

The monkey.—The spinal cord of a *Macacus cynomolgus* was exposed in the upper dorsal region and completely severed, about half a centimetre of its length was removed, and the wound was closed. The animal made a rapid recovery from the operation. Immediately afterwards all reflexes were absent below the section. A few hours later they reappeared in exaggerated form. Next morning the reflexes were absent but reappeared in the afternoon and were nearly normal in amount. The muscles of the hind limbs remained soft and flaccid.

On the morning of the second day after the operation the tendon reflexes were absent but a slight amount of abdominal skin reflex could be obtained. In the afternoon the tendon reflexes were still absent but a reflex was obtained on applying cold water to the sole

of the foot, and also on applying a moderately strong induction current. All the reflexes were, however, much weaker than normal. Saving the complete paralysis of the posterior part the animal appeared to be in good health.

On the three succeeding days, feeble reflexes of all types were obtained from the paralysed parts gradually increasing in strength with the lapse of time, and about a week after the operation the strength of the reflexes had almost increased to normal. But at no period in the course of the experiment after this time were exaggerated reflexes obtained. Two or three days after the operation, commencing atrophy of the muscles of the hind limbs was observed, and at the close of the experiment about fourteen days after the operation the atrophy of these muscles was excessive, although the animal had throughout the greater part of the time a normal appetite and was allowed as much food as he desired.

The animal died of intestinal obstruction, fourteen days after the operation. Post-mortem examination further showed that the cord was cut at the level of the fourth dorsal vertebra.

2. A *Macacus radiatus* was operated upon in a similar fashion and the cord cut as nearly as possible in the same situation. Two hours after the operation, when the animal had completely recovered from the anaesthesia, no reflexes could be obtained from the posterior part, and the muscles of the hind limb were in a relaxed, flaccid condition. In this animal, the reflexes never reappeared at any time during the ten days of observation.

As all reflexes were entirely absent after this period had elapsed and showed no indications of returning, we determined to administer strychnine in order to test whether reflexes could be obtained from the isolated cord.

The results were exceedingly interesting and demonstrated conclusively that the reflex arc was perfectly intact. Five milligrams of sulphate of strychnine were injected subcutaneously. After the lapse of about five minutes the anterior portion of the animal including the fore limbs passed into a strong tetanic spasm, while the posterior portion remained perfectly quiet. About one minute later the posterior portion passed into tetanus independently. The tonic spasms were as usual followed by clonic convulsions which again appeared independently in the two portions of the animal and as before at a later period in the posterior extremities. The animal died from the effects of the strychnine and a post-mortem examin-

ation showed that the spinal cord had been completely separated opposite the fifth dorsal vertebra.

The reflexes were so completely absent in this animal before the administration of the strychnine and the tetanus obtained by the action of the drug so intense, that the experiment suggested to us that the tetanus might be due to direct action of the strychnine on the motor cells of the spinal cord. We accordingly performed the following experiment upon a dog. The lower dorsal and entire lumbar cord were exposed. The cord was completely severed in the lower dorsal region, and all the posterior spinal nerve roots were severed below the point of section. Strychnine was now subcutaneously injected, and it was found that the anterior portion of the animal passed into a tetanic spasm, but that there was not the slightest trace of muscular contraction of the hind limbs; nor could muscular contractions be obtained even when the strychnine was injected into the lumbar cord itself. This negative result conclusively proved that the tetanus obtained in the monkey was purely reflex and hence that the reflex arc was perfectly intact in that animal.

In reviewing our series of experiments, the fact is at once evident that independent spinal reflexes are much more pronounced in lower than in higher forms of animal life. A frog with its spinal cord severed from the brain will re-act with great violence to a slight stimulus. Not so strong, but still decided, are the movements in mammals such as the rabbit or cat while in this condition; but in the monkey or in man they are either very slight or entirely absent. Attention may also be drawn to the important fact that in one of the monkeys experimented upon the reflex activity very gradually returned, and that in the other animal although the reflexes appeared to be entirely absent when tested for by ordinary methods, yet reflex activity must have been present to a slight extent as shown by the re-appearance of the reflexes under the exaggerating influence of the strychnine. These variations in reflex activity can, we believe, only be explained on the supposition that accompanying the changes in relative development of brain and cord in different animals there is a corresponding change in the relationship between these two parts of the central nervous system. As the brain advances in development the spinal cord undergoes a retrograde change and at the same time that it passes more completely under the control of the brain it becomes correspondingly less efficient as an independent reflex centre.

The control of the brain upon the spinal cord will hence vary in character with the stage of development and must not, as is too often the case, be described as a purely inhibitory action. The above series of experiments as well as the results obtained by other workers upon the subject may be more clearly understood by supposing that the influence of the higher centres *regulates* the activity of the spinal reflex centres rather than merely inhibits them.¹

If now this regulating influence be removed and especially if it be suddenly and entirely cut off a decided disturbance in the condition and activity of the spinal cells must result and cause accompanying changes in the reflexes. The direction of such an alteration will obviously vary with the extent to which the motor nerve cells of the reflex arc are played upon by external nerve impulses. If such external nerve impulses reaching the motor cell of the reflex arc from the cerebrum and other nerve centres have previously taken a large part in setting the motor cell into activity, then it is natural to suppose that on the removal of these influences the functional activity of the motor cell will be depressed and hence that the reflex activity will also be weakened; but if the motor cell has chiefly been set in action in a directly reflex manner and has only been controlled to a much slighter extent by impulses arriving from other centres, then its activity will be little influenced by the absence of these external stimuli and its responses to reflex stimulation may actually be increased by their removal. This view of the regulation of reflex activity by cerebral impulses gains some support from the experimental observation recorded above that in the monkey the reflexes tend to reappear and then increase in strength with the lapse of time after the operation. For this recurrence of reflexes would indicate that the cells are gradually recovering from their condition of depressed excitability. Again, if the connection between brain and cord be only slowly interrupted by some gradually developing pathological change, as in Senator's case described above, then it is found that the reflexes never disappear but are on the contrary somewhat exaggerated. This is also in accord with the view expressed above, for a slowly progressive removal of the external nerve impulses would allow a corresponding change of function gradually to take place in the cell and so prevent a disappearance of the reflexes at any stage in the process.

¹ Such a regulating activity might be exercised either as a trophic influence on the motor cells of the reflex arc, or by tonic nerve impulses controlling these cells.

THE ORIGIN OF FIBRINOGEN.

By ALBERT MATHEWS.

[From the Laboratory of Physiology in the Harvard Medical School.]

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INTRODUCTION.

WHAT is the source of the blood proteids? That they are not formed directly out of the proteids of the food is shown by their undergoing relatively little change during fasting and by their failure to increase during the digestion of proteid food. They must be formed somewhere in the body itself. Are they formed by all organs and tissues, or by some one tissue? This question may possibly be answered by following the proteids of the food during absorption and by studying the manner in which the proteids of wasting tissues are converted during fasting into the characteristic proteids of the blood.

The transformation of food proteids into blood proteids has been studied by Salvioli,¹ Hoffmeister,² Neumeister,³ and others. The general result reached by these observers is that the transformation takes

¹ SALVIOLI: Archiv für Physiologie, 1886, p. 98.

² HOFFMEISTER: Archiv für exper. Pathol. u. Pharmacol., 1885, xix, p. 1.

³ NEUMEISTER: *Ibid.*, 1887, xxii, p. 1.

place in the wall of the intestine and stomach. The products of proteid digestion, peptone and albumoses, do not appear as such in the blood. Whether they are transformed into all the blood proteids equally, or chiefly as Neumeister and others have held into serum albumin, is still an open question. Is this transformation brought about by the lymphatic or epithelial tissue of the intestine? Hoffmeister¹ believes that the leucocytes of the lymphatic tissue are pre-eminently active in proteid absorption. He compared the leucocyte, taking peptone from the intestine and carrying it to the tissues, to the red corpuscles carrying oxygen to the tissues. He based his conclusions upon the presence of enormous numbers of leucocytes in the intestinal wall during the absorption of proteid food, upon the proliferation and growth they undergo during proteid absorption, upon the leucocytosis following a proteid meal, and the decrease of the leucocytes in the intestinal area during fasting. Neumeister,² Heidenhain,³ and others have maintained, on the contrary, that the absorption and assimilation of proteids is a function of the epithelial cells of the intestine, the leucocytes playing a subordinate part. In support of this view they cite the facts that peptone is not readily assimilated by the lymph system when injected into the latter; that peptone passing in the blood through the spleen is not appreciably affected by that organ; ⁴ that the mucous membrane of the stomach containing comparatively few leucocytes can transform peptone into an albumin; and, finally, the conclusion derived from Heidenhain's computation that the leucocytes are not present in the intestinal wall in sufficient numbers to account for the transformation of all the proteids of the food.

A study of the manner in which the blood proteids are maintained during fasting will enable us, I believe, to decide with some certainty between these views; at least as regards the final steps in the assimilation. The evidence derived from this source confirms Hoffmeister. During fasting the percentage of proteids in the blood remains practically normal, with the exception of a partial decrease sometimes occurring in the serum albumin (Miescher,⁵ Burckhardt⁶). This

¹ HOFFMEISTER: *Zeitschrift für physiologische Chemie*, 1881, v, p. 151.

² NEUMEISTER: *Zeitschrift für Biologie*, 1888, vi, p. 277.

³ HEIDENHAIN, R.: *Archiv f. d. ges. Physiol.*, 1888, xliii, Sup. Heft., p. 69.

⁴ SHORE: *Journal of physiology*, 1890, xi, p. 559; NEUMEISTER: *Physiologische Chemie*, Jena, 1893, i, p. 252.

⁵ MIESCHER'S *Arbeiten*, Leipzig, 1897, ii, pp. 167 and 318.

⁶ BURCKHARDT: *Archiv für exper. Pathol. und Pharmacol.*, 1883, xvi, p. 330.

shows that the proteids must constantly be produced in the body at this time. They are obviously derived in the long run from the tissues of the body which are wasting away. These tissues are chiefly the skeletal muscles, the glands, and the lymphatic tissue of the intestine.

The manner in which these tissue proteids are converted into blood proteids has never received adequate study. It is clear that the proteids of the decomposing tissues, with the exception of the lymphatic, do not find their way unchanged into the blood, for no strange proteids are to be found there during fasting. The proteids eliminated by these wasting tissues must first be assimilated by some other tissues or organs of the body. These tissues are probably those concerned in the assimilation of proteid food, since in each case the products of the assimilation are the same. Is this assimilation of wasting muscle and gland carried out by the leucocytes, or by the epithelium of the intestine? All known facts speak for the first possibility. Were the intestines the active agents in this transformation the strange proteids should first appear in the blood. No trace of them, however, is to be found here, either by abnormal physiological reactions on the part of the body, or by chemical examination. The conclusion would seem justified that the transformation must be brought about before these proteids reach the blood, in other words, in the lymphatic system. Microscopical study of tissues undergoing rapid decomposition has shown the leucocytes to be the essential factors in the removal and assimilation of such tissues. One has only to recall the rôle of the phagocytes in the removal and assimilation of the wasting tissues of the tadpole's tail or of degenerating muscle and nerve. In these cases the leucocytes are undoubtedly the agents of the absorption and assimilation of the degenerating proteids. During fasting, however, the leucocytes do not penetrate the wasting tissues. We are hence forced to the conclusion either that the proteids set free by disintegrating tissues during fasting are assimilated by some tissue other than the lymphatic, or that the wasting is so gradual as not to cause a local phagocytosis, the leucocytes of the blood and lymph being able to care for these strange proteids as fast as they appear. The well established fact of the phagocytic action of the leucocytes in rapid wasting and the certainty that these proteids must be assimilated before reaching the blood makes the latter explanation the more probable. This is also strongly indicated by the experiments of Buchner, Metchinkoff, and many others, who have introduced strange

proteids and the products of their digestion into various parts of the animal body, and found that they were removed and assimilated by the leucocytes. In any case there is no reason to believe that the assimilation of wasting tissues is carried on by the cells of the intestinal epithelium. Thus the removal of disintegrating tissues, in my opinion, clearly confirms Hoffmeister's hypothesis.

This hypothesis would be strengthened still further could it be shown that the blood proteids are actually derived from the leucocyte, for we should then have evidence that the leucocytes not only absorb and assimilate strange proteids in all parts of the body, but that by their disintegration they liberate in the blood the proteids proper to that fluid. Some evidence in favor of such a source of the plasma proteids has already been urged by Alexander Schmidt. Schmidt¹ showed that in all likelihood the paraglobulin of the blood was set free upon the disintegration of the leucocytes. He obtained from the leucocytes a substance called "cytoglobin." This substance is probably a nucleo-proteid, as is shown by its high content of phosphorus and its reaction to peptic digestion. Schmidt found that cytoglobin digested in an alkaline medium such as the blood passed by a series of changes into a phosphorus-free substance giving the reactions of the paraglobulin of the blood. Schmidt believed that the transformation proceeded one step further, converting the paraglobulin into fibrinogen. His idea that paraglobulin is a derivative of a nucleo-proteid, possibly nucleo-histon, set free on the decomposition of the leucocytes receives a certain degree of confirmation in Mörner's² observation that paraglobulin contains a carbohydrate radicle. Kossel and Neumann³ have shown that nucleo-histon contains a carbohydrate radicle, as is indicated by its yielding levulinic acid when digested with acids. Mörner's discovery that paraglobulin under similar treatment also yields a reducing substance confirms Schmidt. Both bodies, moreover, are acids.⁴ Indirect evidence, also, leads to the conclusion that upon decomposition of the leucocytes a substance free from phosphorus- and nuclein-bases must be set

¹ SCHMIDT: *Zur Blutlehre*, Leipzig, 1892, p. 167.

² MÖRNER: *Centralblatt für Physiologie*, 1893, vii, p. 581.

³ KOSSEL and NEUMANN: *Berichte der deutschen chemischen Gesellschaft*, 1894, ii, p. 2215.

⁴ HAMMARSTEN: *Lehrbuch der physiologischen Chemie*, dritte Auflage, 1895, p. 538.

free. It is fairly certain that upon leucocytic disintegration the xanthin bases and phosphoric acid are set free, as is shown by their increased excretion. These substances are probably derived from the nucleo-histon of the leucocyte. After these radicles have been split off there remains unaccounted for the rest of the nucleo-histon molecule. This should be a proteid containing a carbohydrate group. It is just this radicle which, according to Schmidt, paraglobulin should be. Hence there is some reason for believing that the paraglobulin of the blood is derived from the leucocytes on their decomposition, and that probably it represents the chromatin of their nuclei after the nuclein bases and phosphoric acid have been split off. The series of changes this body undergoes may account for Hammarsten's¹ conclusion that paraglobulin is a mixture of several proteids.

The origin of the serum albumin of the blood is less clear. Two facts indicate, however, that it originates in the same cells as the paraglobulin. On the one hand the quantitative relationship of serum albumin to paraglobulin in any given blood is fairly constant, not varying greatly even in fasting. Had the two bodies separate origins they probably would run different courses. On the other hand both Lilienfeld and Miescher² isolated from the leucocytes a substance having the properties of serum albumin. Its solubility and coagulation temperature were identical with those of the albumin. Did serum albumin represent simply transformed food proteid it should diminish rapidly on fasting. This it does not do.

The remaining proteid of the blood is fibrinogen, concerning the origin of which little is known. Wooldridge obtained from many tissues substances which he termed fibrinogens, but with the exception of his first or true fibrinogen these substances have been shown to be nucleo-proteids yielding fibrin ferment but not fibrinogen. His work, therefore, throws little light on the origin of the fibrinogen of the blood. Schmidt,³ in his last treatise on the blood, regards the para or serum globulin of the blood as the immediate precursor of fibrinogen. Schmidt, as has been said, derived the paraglobulin from the cells chiefly of the lymphatic tissue. He believed, however, that all cells contained cytoglobin, and contributed thus to the formation

¹ HAMMARSTEN: *loc. cit.*, p. 104.

² MIESCHER: Hoppe-Seyler's Medicinisch-chemischen Untersuchungen, iv, p. 441.

³ SCHMIDT: Zur Blutlehre, Leipzig, 1892, p. 167.

of paraglobulin and fibrinogen. Schmidt was unable to convert paraglobulin into fibrinogen. Such a conversion never takes place outside the body, however rich in fibrin ferment a paraglobulin solution may be, and Schmidt accordingly was forced to assume that the transformation was brought about by the body cells. Schmidt believed that fibrinogen was derived from paraglobulin for two reasons: First, an *a priori* objection to supposing the long series of changes culminating in paraglobulin to be paralleled by a second series culminating in the production of fibrinogen; and, second, the fact that the addition of paraglobulin to a solution of fibrinogen increases the amount of fibrin to be obtained from the latter. He believed the latter fact indicated that some of the paraglobulin had been converted into fibrinogen. Schmidt's first reason, being purely *a priori*, may be dismissed. For the second, the possibility of a direct union of the paraglobulin and fibrin, thus increasing the weight of the latter, is not guarded against in Schmidt's experiments. This explanation of the increase of weight of the fibrin caused by Schmidt's fibrinoplastic substances, a theory first held by Schmidt himself, is supported by the observations of Kossel¹ that protamin and histon unite with albumin in slightly alkaline solution, and those of Kutscher² that nucleinic acid and some of the albumoses react similarly. Since paraglobulin is possibly a derivative of nucleo-histon, and if so should contain portions of the radicles of both nucleinic acid and histon, this idea of a direct union between fibrin and paraglobulin is not lacking in probability. While provisionally accepting, therefore, Schmidt's conclusion as to the origin of paraglobulin in the leucocytes, his conclusion as to the origin of fibrinogen in the paraglobulin cannot be accepted.

Rauschenbach³ observed that the addition of leucocytes or pus cells, or extracts of these cells, to the blood increased the output of fibrin, and hence concluded that they gave rise directly to fibrinogen. His work is open, however, to the same objection as Schmidt's.

Dastre has recently emphasized the fact that a consumption and production of fibrinogen are constantly going on, and has endeavored to class certain organs as fibrinogen producers, and others as fibrinogen consumers. He considers the lungs, intestine, and skin as

¹ KOSSEL: Zeitschrift für physiologische Chemie, 1896, xxii, p. 186.

² KUTSCHER: *Ibid.*, 1897, xxiii, p. 117.

³ RAUSCHENBACH: Inaugural Dissertation, Dorpat, 1883. See SCHMIDT: *loc. cit.*, pp. 55 and 177.

producers of fibrin, — the kidney, liver, and other organs as consumers. In thus sharply defining the problem and emphasizing the nutritive rôle of fibrinogen Dastre¹ has rendered a distinct service. The evidence, however, that certain organs are producers and others consumers is not conclusive. This evidence will be considered for each organ in its turn. Dastre found that the arterial blood contained more fibrinogen than the total blood, thus indicating that the venous blood in general was poorer in fibrinogen, and hence that fibrinogen was consumed in the tissues. Lehmann long ago observed that the blood of the mesenteric veins was richer in fibrinogen than the arterial blood. This fact is the most valuable indication we have of the origin of fibrinogen.

METHOD.

Dastre found, as Bizzozero had already independently discovered, that it is possible to defibrinate totally the blood of dogs by successive bleedings, defibrinations, and reinjections. By this means the fibrinogen may be completely removed from the blood. The dogs bore the operation well and re-formed the fibrinogen. This observation of Dastre's suggested the possibility of discovering the origin of fibrinogen by extirpating one organ after another in the defibrinated animal and observing the influence of the extirpation on the re-formation of the fibrinogen. This method has been followed for the kidney, spleen, pancreas, brain, reproductive organs, and intestine, and in determining the influence of food. The method is not applicable to all organs, and has been supplemented by a comparison of the fibrinogen content of the arterial and venous blood of various organs, and by perfusion experiments with defibrinated blood to determine whether certain organs have the power of re-forming fibrinogen. Cats have been used almost exclusively. A few experiments were performed on dogs. The animals were in all instances anaesthetized with ether, or a mixture of ether, chloroform, and alcohol; the funnels, filter, cannulas, and beakers were rinsed out with boiling water before each defibrination, to sterilize them as far as possible. Ordinary antiseptic precautions were taken.

In defibrination the operation was the following: The animals were weighed, the total blood calculated at one thirteenth the body weight, and a quantity equal to one third the total blood thus ascertained drawn from the carotid or femoral artery. Sometimes somewhat

¹ DASTRE: *Archives de physiologie*, 1893, xxv, pp. 327, 628.

more could be taken, but generally somewhat less. In all cases blood was drawn until respiration was strongly affected. This blood was defibrinated by whipping, filtered through gauze into a warmed double funnel, and reinjected slowly (100 c.c. in five minutes) into the femoral or jugular vein. It is necessary to reinject the blood with care, otherwise thrombosis may result. Several cats were lost in this way. Confirming Schmidt's observations, I found the danger of intravascular clotting much greater in cats than in dogs. After the first reinjection a pause of four to ten minutes was made to allow the reinjected blood to mix well with that in the body. A third of the blood was again drawn, defibrinated, and reinjected. This process was repeated six or eight times. Little or no fibrin can be obtained from cats after the fifth or sixth drawing. Dogs, however, yield fibrin after the eighth. In cats the fibrin ferment of the blood almost totally disappears, and the number of leucocytes enormously diminishes after the third or fourth drawing. As a consequence clotting is exceedingly slow, and the blood must be whipped ten minutes or more; and then, as Dastre also observed, clotting is apt to take place in the venous cannula. I found it advisable, hence, to add to the fourth and all subsequent drawings a few cubic centimetres of the normal saline washings from the first masses of fibrin. By this addition of fibrin ferment what fibrin there is in these last drawings may be rapidly removed. These facts show that in the cat at least, contrary to Dastre's observation on the dog, fibrin ferment set free in the defibrinated blood is rapidly removed from the blood by the body. By drawing one third of the blood each time there is removed one third of the fibrin in the first defibrination, $\frac{2}{9}$ in the second, $\frac{4}{27}$ in the third, $\frac{8}{81}$ in the fourth, $\frac{16}{243}$ in the fifth, and $\frac{32}{729}$ in the sixth. In all 0.91 per cent of the total fibrin. A correction must hence be made of 0.1 per cent in all determinations of the total fibrin by this method. The fibrin was rubbed in a mortar with successive portions of water and normal saline solutions until white, the washings decanted through a weighed filter, washed free from chlorine, extracted with hot 95 per cent alcohol and ether, dried at 110° , and weighed. By rubbing in the mortar the fibrin can be rapidly cleansed, as shown by microscopic examination.

The determination of the percentage of fibrin in the various regions of the vascular system was similarly made, the fibrin being rubbed thoroughly in a small mortar, and thereafter treated according to Hoppe-Seyler.

In the perfusion experiments the defibrinated blood of one cat was perfused through the organ of another.¹

The following experiments were made to show whether the amount of fibrin actually recovered corresponds to that computed for the whole blood from a determination of the percentage in arterial blood.

Experiment XXIX. Per cent of fibrin in carotid blood, 0.35. Weight of cat, 3.052 grams. Total blood of cat, 234 grams. Total fibrin calculated from arterial per cent, 0.7000 gram. Total dried fibrin actually recovered, 0.5766 gram.

Experiment XXVII. Per cent of fibrin in carotid blood, 0.35. Weight of cat, 2.580 grams. Total blood of cat, 198 grams. Total fibrin calculated from arterial per cent, 1.0494 grams. Total dried fibrin actually recovered, 0.9327 gram, with $\frac{1}{10}$ correction, 1.0377 grams.

Experiment V. Per cent of fibrin in carotid blood, 0.37. Weight of dog, 8.225 grams. Total blood of dog, 633 grams. Total fibrin calculated from arterial per cent, 2.342 grams. Total dried fibrin actually recovered, 2.0234 grams.

Experiment LVIII. Cat. Per cent of fibrin before defibrination, 0.139. Fifteen minutes after the end of defibrination the blood contained 0.008 per cent of fibrin. By calculation there should have been present $\frac{1}{10}$ of 0.139, or 0.014 per cent.

I have never been able to render the blood completely incapable of coagulation by this method. It eventually clotted in all cases examined, though the period before clotting set in was generally prolonged from thirty minutes to several hours. As the blood, however, may be rendered practically incapable of coagulation under ordinary circumstances by this method, the operation requiring some forty-five minutes only and not injuring the cat, it would seem that the method might be used with advantage in experiments which would be interfered with by clotting. The foregoing results are in accord with those of Dastre that the total defibrination always yields an amount of fibrin less than that calculated to be present from the fibrin content of arterial blood. The correspondence is sufficiently close, however, to show that the method will remove approximately all the fibrinogen in the blood.

THE PHYSIOLOGICAL EFFECTS OF DEFIBRINATION.

Cats and dogs survive defibrination without great difficulty; cats more easily than dogs. The only symptoms detected were weakness,

¹ I am indebted to Dr. A. Cleghorn for assistance in the perfusions.

loss of appetite, and drowsiness, all of which might be attributed to the prolonged etherization. In a day or so cats recover entirely. In a number of instances I have defibrinated cats three or four times at intervals of several days, and the animals remained apparently normal several weeks after the operation, when they were used for other experiments. Dastre states that not more than one fifth the blood can be drawn at one time from dogs without serious danger, but cats readily permit a withdrawal of one third without evil effects. The rectal temperature was taken in one cat during successive defibrinations lasting in all for three hours. The temperature was 39° before defibrination; after the first reinjection, 38.2° ; after the third, 37.1° ; after the fourth, 36.5° ; after the sixth, 37.5° , where it remained. Hence defibrination is not accompanied by any marked disturbance of temperature. The only change noticed in the abdominal organs after defibrination was an intense vaso-dilation in the suprarenal bodies.

RAPIDITY OF RE-FORMATION OF THE FIBRIN IN NORMAL CATS.

Dastre reports two experiments in dogs which indicate a very rapid re-formation of fibrinogen. In one case, the first five hundred grams of blood yielded 0.600 gram of dried fibrin. The blood was then defibrinated in eleven drawings. Three hours and a half later five hundred grams yielded 0.269 gram of dried fibrin. Twenty-four hours after defibrination he states that the total fibrin had surpassed by one third the amount present in the first defibrination. I am inclined to doubt the correctness of this result, however, for the reason that if it were true that such a rapid regeneration of fibrin took place, in total defibrination, extending as it does over three to six hours, a far larger percentage of fibrin should be obtained than would be indicated by the percentage in the arterial blood. Certainly in the cat no such rapid re-formation takes place, as is shown by the protocols which follow. In the dog, however, two days suffice to more than replenish the supply of fibrinogen.

Experiment XIV. Cat. First defibrination Feb. 9, 11 A.M.; total fibrin, 0.1690 gram. Second defibrination Feb. 9, 3 P.M.; total fibrin, 0.000 gram. Third defibrination Feb. 11; total fibrin, 0.2200 gram.

Experiment LIV. Cat. First defibrination April 27, 11 A.M.; per cent of fibrin, 0.254. Second defibrination April 27, 5 P.M.; per cent of fibrin, .094.

Experiment I. Cat. First defibrination Jan. 18; total fibrin, 0.4723 gram. Second defibrination (spleen out) Jan. 19; total fibrin, 0.1458 gram.

Experiment II. Cat. First defibrination Jan. 21; total fibrin, 0.5062 gram. Second defibrination Jan. 23; total fibrin, 0.4375 gram.

Experiment VIII. Cat. First defibrination Feb. 3; total fibrin, 0.2230 gram. Second defibrination Feb. 5; total fibrin, 0.2293 gram. Third defibrination Feb. 8; total fibrin, 0.2605 gram.

Experiment IX. Cat. First defibrination Feb. 3; total fibrin, 0.5230 gram. Second defibrination Feb. 4; total fibrin, 0.3220 gram.

Experiment X. Cat. First defibrination Feb. 4; total fibrin, 0.2950 gram. Second defibrination (spleen out) Feb. 6; total fibrin, 0.2400 gram.

Experiment XI. Cat. First defibrination Feb. 5; total fibrin, 0.3820 gram. Second defibrination (spleen out) Feb. 16; total fibrin, 0.5735 gram. Third defibrination Feb. 19; total fibrin, 0.4405 gram.

Experiment XII. Cat. First defibrination Feb. 6; total fibrin, 0.2402 gram. Second defibrination Feb. 9; total fibrin, 0.7546 gram.

Experiment XVI. Cat. First defibrination Feb. 12; total fibrin, 0.5816 gram. Second defibrination Feb. 13; total fibrin, 0.1600 gram. Third defibrination Feb. 15; total fibrin, 0.4355 gram. Fourth defibrination Feb. 18; total fibrin, 0.7405 gram.

Experiment II. Dog. First defibrination Nov. 2; total fibrin, 1.0650 grams. Second defibrination Nov. 6; total fibrin, 2.7542 grams.

Experiment IV. Dog. First defibrination Nov. 14; total fibrin, 2.3660 grams. Second defibrination (spleen out) Nov. 16; total fibrin, 3.2405 grams.

From these figures it is clear that the fibrinogen is only slowly in normal circumstances re-formed to its usual amount. In twenty-four hours it is still below the normal. In from thirty-six to seventy-two hours it reaches again its normal amount, and often rises above the normal, as Dastre and Magendie have observed.

ORIGIN OF FIBRINOGEN.

Is fibrinogen derived from paraglobulin? — Schmidt believed that the fibrinogen was derived from paraglobulin. Dastre has expressed a somewhat similar view in stating that the new fibrin formed in defibrinated animals more nearly approaches a globulin than that generally found in them, being more soluble in dilute saline solutions. I cannot confirm the last observation. It may be mentioned that Dastre¹ himself has shown recently that normal fibrin is more soluble in dilute salt solution than was previously supposed. No transformation of paraglobulin into fibrinogen takes place outside the body. If the fibrinogen be once thoroughly removed, no more is generated even

¹ DASTRE: Archives de physiologie, 1894, xxvi, p. 919.

though the blood be kept well oxygenated at the body temperature until putrefaction sets in. This forced Schmidt to the conclusion that the transformation was brought about by the cells of the body. This theory can be tested only by the perfusion of living tissues with defibrinated blood rich in paraglobulin. Schmidt's own observations gave throughout a negative result. The body of a frog was well washed out with saline solution, and then perfused for many hours with defibrinated horse's blood; not a trace of fibrinogen formation occurred. A tortoise heart was also washed as clean as possible from normal blood and filled with defibrinated blood. After twenty-four hours the heart was opened. Very minute clots were found, due, Schmidt thought, to the admixture of normal blood which it was impossible to wash out completely. On the removal of these clots no more formed. In one of Professor Porter's experiments a tortoise heart was kept beating with an artificial circulation of defibrinated blood for twenty hours. There was no indication of clots in it at the end.

In the perfusion of mammalian muscle it is a common experience that the first blood flowing from it clots. Schmidt believed, however, that this fibrinogen was derived from the normal blood left in the muscle, and was not due to a conversion of paraglobulin to fibrinogen. I undertook a perfusion experiment on the hind limb of a cat to determine whether such a transformation of paraglobulin to fibrinogen occurred in the tissues of the limb. A cat was first defibrinated so far as possible, and about three hours later was perfused. Its blood at that time clotted loosely. Defibrinated blood of a second cat was run into the femoral artery and recovered from the femoral vein. The first blood from the vein clotted loosely. The fibrinogen came in this case from an admixture of systemic blood coming to the limb by undiscovered anastomoses. This was shown by the fact that a slight flow of blood from the femoral vein persisted after cutting off the perfusion, and by the fact that the bulk of the perfused blood augmented somewhat. The cat was then killed by ether, and when the cat's own circulation ceased the fibrin no longer appeared in the perfused blood. After the death of the cat the two hundred cubic centimetres of blood were kept circulating through the living limb for six hours, the whole quantity passing through twenty-four times. At the close of the experiment a portion of the blood was set one side. No clot appeared in it. To a second portion the normal salt washings of a fibrin clot were added. This portion also

remained completely fluid. The serum of a third portion was filtered, diluted with a similar volume of 0.8 per cent salt solution, and added to an equal volume of a saturated solution of sodium chloride. Had fibrinogen been present it should have been precipitated. The mixture remained perfectly clear.

This experiment proves clearly that none of the tissues of the cat's leg, *i. e.* endothelium of the blood vessels, skeletal muscles, skin, connective tissue, or bone, is capable under the conditions of the experiment of transforming paraglobulin into fibrinogen. It shows also that these tissues do not produce fibrinogen. The experiments of Schmidt enable us to throw out the heart, and in the many perfusions of the kidney I can find no reference to the reappearance of fibrinogen. The transformation of paraglobulin does not take place, therefore, outside of the body, nor in the tissues just mentioned. In the complete absence of any evidence of such a transformation, the paraglobulin origin of fibrinogen must be regarded with scepticism, if not altogether relinquished. The observations of Morner that paraglobulin yields on decomposition a reducing substance, which fibrinogen does not yield, would indicate, if confirmed, that fibrinogen and paraglobulin are very different substances.

Does the spleen form fibrinogen? — The parallelism existing between uric acid excretion and the fibrinogen content of the blood, discussed on page 76, suggested that the organ might hold the same relation to fibrinogen that Horbaczewski has shown it to hold to uric acid. The following experiments indicate, however, that the spleen is not an important source of fibrinogen either in the dog or cat. These animals when deprived of the spleen are able to re-form the fibrinogen in a normal time. After the removal of the spleen the percentage of fibrinogen in the blood does not diminish as it would do were this organ an important source.

Experiment V. Cat. Weight, 2,760 grams. Spleen extirpated Jan. 26. On Jan. 28 the blood was defibrinated, yielding 0.8088 gram of dried fibrin, or 0.38 per cent. This is higher than the normal fibrin content of cats, and shows that in the forty-eight hours elapsing between extirpation and defibrination no diminution in fibrinogen had taken place.

Experiment XI. Cat. Weight, 3,900 grams. Defibrinated Feb. 5; total fibrin recovered, 0.3820 gram; spleen extirpated at close of defibrination; cat recovers quickly. Second defibrination on Feb. 16; weight of cat, 3,657 grams; total fibrin recovered, 0.5755 gram. Third defibrination on Feb. 19; weight of cat, 3,651 grams; total fibrin recovered, 0.4405 gram. Fourth defi-

brination on March 22; weight of cat, 3,686 grams; per cent of fibrin in carotid blood, 0.22; total fibrin not weighed; suppuration ensues. On March 26 cat weighed 3,250 grams. Per cent of fibrin in carotid blood had risen to 0.54. Cat still alive and well on June 1.

Experiment X. Cat. Weight, 4,039 grams. Defibrinated at 9 A.M., Feb. 4; total fibrin recovered, 0.2950 gram; spleen extirpated at the close of the defibrination; cat recovers quickly; no suppuration or inflammation. Second defibrination on Feb. 6, 9 A.M.; total fibrin recovered, 0.2400 gram. Cat died on the 8th; cause undetermined.

Experiment IV. Dog. Terrier. Weight, 12 kilos. Morphine subcutaneously; A. C. E. mixture. Defibrinated at 9 A.M., Nov. 14; total fibrin recovered, 2.3660 grams, or 0.29 per cent; spleen removed at close of defibrination. Second defibrination Nov. 16; blood coagulates only after one hour and thirty minutes; well marked buffy coat; total fibrin recovered, 3.2405 grams, or 0.40 per cent. Dog died on the 17th. Autopsy showed large, white, solid clots of fibrin in the right heart and pulmonary arteries.

From the foregoing experiments it is plain that the spleen is not the sole, or an important, organ of formation of fibrinogen.

The pancreas. — To determine whether the pancreas is an essential organ of fibrinogen formation, two experiments were tried. Each gave a decisive result.

Experiment XLII. Cat. Female. At 10 A.M., April 13, the carotid blood contained 0.139 per cent of fibrin. The pancreas was removed without hemorrhage immediately thereafter. Cat bore operation well. At 10 A.M., April 14, blood drawn from the inferior vena cava contained 0.582 per cent of fibrin.

In this experiment the fibrin content of the blood had increased in the absence of the pancreas in twenty-four hours over seventy per cent.

Experiment LVIII. Cat. Female. Defibrinated and pancreas with spleen extirpated without hemorrhage. Before defibrination at 9 A.M., the carotid blood contained 0.139 per cent of fibrin. At the end of defibrination, immediately after removal of the spleen and pancreas at 10.30 A.M., the blood yielded on long standing 0.008 per cent of fibrin. At 5.30 P.M., seven hours later, the carotid blood contained 0.119 per cent of fibrin. This blood, after removal of the fibrin, showed a remarkably thick layer of white corpuscles above the red.

This experiment, showing that an animal is capable of bringing its fibrin almost to the normal amount in seven hours after defibrination in the complete absence of both spleen and pancreas, clearly dem-

onstrates that neither of these organs is essential to fibrinogen formation.

The central nervous system.—Although it is practically impossible to cut off altogether the circulation of the blood through the whole nervous system, the following experiment, in which the brain and upper part of the spinal cord were eliminated without marked decrease in the power of the animal to re-form fibrinogen, indicates that this tissue is not essential to fibrinogen formation.

Experiment LIII. Cat. Male. Defibrinated thoroughly from 9-11 A.M. Blood at the end contained 0.007 per cent of fibrin, yielded on twenty-four hours' standing. After defibrination the abdominal aorta was ligatured immediately below the kidneys. Thereafter one renal artery, the left subclavian, and the innominate arteries were tied. The blood was thus confined chiefly to the abdominal and intercostal muscles and the intestinal area, heart, and lungs. Artificial respiration with warm air was kept up, and the cat was wrapped in cotton-wool and kept in a warm place. The circulation lasted seven hours. *Rigor mortis* was pronounced in the limbs and neck muscles, but was absent in the thorax. At the end of seven hours immediately following stopping of the heart, the abdomen was opened and blood drawn from the upper end of the inferior vena cava. This blood clotted normally, and yielded on whipping 0.072 per cent of fibrin. This is practically normal, as a normal control cat (*Experiment LIV*, page 62), seven hours after defibrination, showed a fibrin percentage of only 0.09 in the carotid blood, which is generally 0.01-0.02 per cent richer in fibrin than the blood of the inferior vena cava.

This experiment, showing an almost normal power of fibrinogen formation in the absence of the greater part of the nervous and muscular tissues of the body, strongly indicates that these tissues are not essential formers of fibrinogen.

The kidneys.—The kidney is ranked by Dastre as a fibrinogen-consuming organ. His conclusion rests on the erroneous observations of Claude Bernard, Brown-Séquard, and others that the blood in the kidney vein does not clot and hence contains no fibrinogen.¹ That the kidney is not an essential former of fibrinogen is indicated by the recent observations of Herter and Wakeman, who found regularly in dogs after kidney extirpation a very great increase in the percentage of fibrin in the blood. In several instances the percentage of fibrin rose from 0.3 to 0.7.

The following experiments on the cat indicate that the kidney is

¹ FLEISCHHAUER: Eckhardt's Beiträge, v, p. 94; HERTER and WAKEMAN: Journal of experimental medicine, 1899, iv, p. 117.

not an important fibrinogen producer, since its removal after defibrination leaves the cat still able to re-form its fibrinogen.

Experiment XXVI. Cat. Weight, 3.147 grams. Defibrinated March 9, 9 A.M.; total fibrin recovered, 0.6562 gram; kidneys extirpated at the close of defibrination. Second defibrination twenty-four hours later; fifty-four grams of blood yielded 0.1925 gram of fibrin, or 0.35 per cent. The cat died during the reinjection of the defibrinated blood. Multiplying the 0.1925 gram by 3, to give the total fibrin, would indicate a total fibrin content of 0.5775 gram.

Experiment XXIX. Cat. Weight, 3.052 grams. Drew 20.6214 grams of blood from the femoral artery; per cent of fibrin, 0.27; kidneys extirpated; leucocytes, 23,200 per cubic millimetre. Twenty-four hours later, leucocytes 40,000 per cubic millimetre; per cent of fibrin, 0.35; total fibrin recovered on defibrination, 0.5190 gram.

In Experiment XXIX the same increase in the per cent of fibrin observed by Herter and Wakeman after kidney extirpation in dogs is to be seen. These experiments, in connection with the observations of Herter and Wakeman, justify the conclusion that the kidneys are not an essential source of fibrinogen, since after their removal the fibrinogen in the blood increases, and since fibrinogen may be re-formed in a normal manner in their absence. This conclusion is further supported by the fact that the blood of the inferior vena cava above the kidneys contains less fibrinogen than the carotid blood.

The muscles. — It is obviously impossible to determine by extirpation experiments the rôle of the muscles, the skin and blood-vessel endothelium as fibrinogen producers. Beside Experiment LIII, page 67, in which the muscles of the limbs, neck, head, and part of the abdomen were thrown out of the circulation without loss of fibrinogen-forming power, I have tried a single perfusion experiment. This experiment has already been given at length on page 64. It shows clearly that the muscles, skin, connective tissue, and endothelium of the blood vessels are incapable of producing fibrinogen. This result is borne out by the determinations of the fibrin content of the arterial and venous blood of the extremities given later. These determinations show that fibrinogen is less in the venous than in the arterial blood.

The reproductive organs. — The formation of fibrinogen by the reproductive organs is improbable, owing to the fact that fibrinogen is formed both in males and females. No loss of clotting power of the blood in castrated animals has been recorded. To determine the point definitely, a castrated cat was examined. (See under Kidney

Experiment XXVI.) In this animal the total fibrin on the first defibrination was 0.6562 gram, or 0.27 per cent. After kidney extirpation 54 grams of blood yielded 0.1925 gram dry fibrin, or 0.35 per cent. The cat in Experiment LV had been castrated at an unknown period, certainly several weeks before the operation; his blood contained, however, 0.152 per cent of fibrinogen, an entirely normal amount.

These facts show that the reproductive organs are not essential to the formation of fibrinogen.

The lungs and liver. — The lungs are considered by Dastre¹ to be generally consumers, but occasionally producers, of fibrinogen. His observations, with the exception of a single experiment, show that the blood of the pulmonary vein contains always somewhat less fibrinogen than the pulmonary artery. In view of the loss of water by the blood in the lungs the contrary effect might have been expected. From Dastre's experiments, the conclusion may be drawn that the lungs consume fibrinogen. Further perfusion experiments are necessary to establish this.

The old observations of Lehmann, Bernard, and others on the non-coagulability of the hepatic blood and the small amount of fibrinogen in the hepatic as compared with the portal blood are inexact. Dastre believes the liver to be a consumer of fibrinogen. As extirpation of the liver after defibrination is practically impossible, recourse must be had to perfusion experiments. These I have not yet carried out. The fibrinogen-consuming or -producing powers of the liver must hence be left for the time in abeyance.

The intestine. — I first endeavored to ascertain whether fibrinogen was formed in the small intestine by ligaturing the superior and inferior mesenteric arteries and the mesenteric veins, and examining the fibrin content of the blood before and after tying the ligatures. The results were unsatisfactory, as the cats lived but from two to four hours, and post-mortem examination showed the circulation not to have been entirely cut off. The fibrin content did not materially alter under these circumstances. In Experiment XLIV it fell from 0.343 per cent to 0.288 per cent; in Experiment XLVIII it fell from 0.261 per cent to 0.247 per cent; but in Experiment LII it rose from 0.279 per cent to 0.335 per cent. If the intestines were the sole producers of fibrinogen, one would expect under such circumstances a distinct fall in the fibrin content of the blood.

¹ DASTRE: *Archives de physiologie*, 1893, xxv, p. 628.

A comparison of the arterial and venous blood of the intestine yields a more interesting result. The venous blood of the body generally is somewhat poorer in fibrinogen than the arterial. Thus Dastre¹ found that blood from the inferior vena cava in dogs contained less fibrinogen than the carotid blood. The following figures show this to be the case also in the cat. This indicates that the fibrinogen is removed from the blood either in the tissues of the lower limbs, trunk, or kidney. Dastre has also observed that in passing through the lungs the blood as a rule appears to lose rather than gain fibrinogen. The blood of the pulmonary vein is generally no richer in fibrin than that of the artery, but on the contrary somewhat poorer. It is clear that somewhere in the course of the blood through the body fibrinogen must be added to the blood, or the percentage of fibrinogen would very rapidly fall. An examination of the blood of the mesenteric vein before it is joined by blood from either the pancreas or the spleen shows the intestine to be the fibrinogen-producing area.

It was long ago observed by Lehmann that the blood of the mesenteric vein contained more fibrinogen than the carotid blood. I can find but one specific determination, however, and in this the difference between the two bloods was too marked to be normal, being about 0.3 per cent. The following determinations show Lehmann's conclusion, however, to be correct. The blood was generally drawn first from the carotid artery, immediately thereafter from the mesenteric vein, before its junction with the splenic, and at once thereafter from the inferior vena cava either above or below the kidney. Some cats were fasting, others in full digestion. In two cases the blood was drawn from the inferior vena cava before it was taken from the mesenteric veins. In Experiment XXXIV the mesenteric vein was not ligatured before the introduction of the cannula, so that the blood flow was at no time interrupted. In the other cases the blood flow was interrupted for from thirty seconds to a minute and a half. The result obtained was the same in all cases in spite of the variations in the procedure employed, with the single exception of Experiment XXXIX. In this case the cat was in full digestion, the blood issuing scarlet from the mesenteric vein. In this cat the fibrin content of the mesenteric vein was the same as that in arterial blood.

The foregoing figures, showing the blood of the mesenteric vein to be richer in fibrinogen than the arterial blood, in distinction from all

¹ DASTRE: *Archives de physiologie*, 1893, xxv, p. 686.

Experiment.	Per cent of Fibrin.		
	Carotid.	Mesenteric.	Vena cava.
XXXIII	0.175	0.228
XXXIV	0.246	0.268	0.228
XXXV	0.307	0.316	0.299
XXXVI	0.209	0.238	0.170
XXXVII	0.375	0.398	0.359
XXXVIII	0.218	0.229	0.160
XXXIX	0.216	0.213
XLIX	0.237	0.229

other venous blood, indicate that fibrinogen is added to the blood in its passage through the wall of the intestine.

There are several large lymphatic glands on the course of the mesenteric veins, and the possibility suggested itself that the fibrinogen was derived from these, particularly as an old observation of Tiedemann and Gmelin showed that the lymph of the horse after passing these glands was richer in fibrinogen. Before the glands were reached it contained but a trace of fibrinogen, but after passing them 0.31 per cent. The following experiment showed conclusively, however, that these glands in the cat are not essential to the formation of fibrinogen:—

Experiment XLI. Cat. Female. At 9 A.M., April 10, the carotid blood contained 0.164 per cent of fibrin. All the visible lymphatic glands in the mesentery were carefully removed without hemorrhage. In all 5.04 grams of moist glands were removed. The operation ended at 11 A.M. At 4.30 P.M., 12.9235 grams of blood were drawn from the carotid. It contained 0.220 per cent of fibrin. On April 12, at 9 A.M., the cat was doing well, but suppurating somewhat from the abdominal wound; 12.1505 grams of blood from the carotid contained 0.542 per cent of fibrin. On April 13, at 9 A.M., another portion yielded 0.472 per cent of fibrin. For the seat of fibrin formation in the intestinal area, we must hence look elsewhere than to the mesenteric glands.

I endeavored to obtain decisive evidence that fibrinogen is produced in the intestine by extirpating the intestine after defibrination.

Experiments in this direction are not completed. I have tried in all five experiments. In all of them the intestine, from a little below the pylorus to the rectum, was removed after defibrination. In the first three experiments the cats died in about three hours, — too short a time to give a decisive result. In all three cases, however, the blood had entirely lost the power of coagulation, and no trace of fibrinogen could be detected. The following two cats lived somewhat longer, and gave a more conclusive result: —

Experiment LIX. Cat. May 9. Fibrin per cent before defibrination, 0.163. Defibrinated. At the end of defibrination the intestine was extirpated without loss of blood. A sample of the carotid blood drawn immediately thereafter clotted very slowly, and yielded on long standing 0.007 per cent of fibrin. The cat lived four hours after the removal of the intestine; 10.276 grams of blood drawn immediately after death from the inferior vena cava remained completely fluid even after twenty-four hours. The normal salt washings of a fresh clot of fibrin induced no clotting in it.

In this cat not only had no fibrinogen been re-formed, but that left there after defibrination had completely disappeared from the blood.

Experiment LX. Cat. Male. Before defibrination the carotid blood contained 0.182 per cent of fibrin. After defibrination the intestine was extirpated without bleeding. The circulation through the stomach, pancreas, spleen, and a small portion of the intestine attached to the pylorus remained. After extirpation the blood contained 0.01 per cent of fibrin. The cat was wrapped in cotton-wool and kept in a warm place. It lived under its own respiration for five hours after removal of the intestine, and for one hour longer the circulation persisted under artificial respiration; 12.0845 grams of blood drawn from the heart end of the inferior vena cava immediately after cessation of the heart-beat yielded on standing 0.0050 grams of fibrin, or 0.041 per cent.

In other words, in the absence of nearly the whole of the small intestine the cat had added 0.03 per cent of fibrinogen to the blood. This is from one third to one half that which the normal cat can re-form in the same time. Whether this slight power of re-formation was due to the parts of the intestine not extirpated, or whether it signifies that fibrin might be formed in other parts of the body could the animal be kept alive, is not clear. Further experiments are necessary to decide this point. At any rate, the behavior of the cat without the intestine is, as regards power of re-forming fibrinogen, in striking contrast to cats deprived of reproductive organs, spleen, pancreas, or kidneys. The absence of these other organs seems to

stimulate the fibrinogen-forming power, whereas removal of the intestine diminishes it, or obviates it altogether.

Although the observations are not altogether conclusive, I believe they justify, nevertheless, the tentative conclusion that fibrinogen is formed in the wall of the intestine. The possibility at once suggests itself that if this is the case it may possibly be formed by a direct transformation of the proteid of the food.

Is fibrinogen derived directly from the food?—Although the fact that the blood retains its proper proportion of fibrinogen during fasting makes the origin of fibrinogen in the food proteid unlikely, decisive experiments were necessary to prove that certainly the presence of proteid food is not a necessary condition for the production of fibrinogen. Cats were allowed to fast for varying periods of time, then defibrinated, and their power of re-forming fibrinogen determined. As the following figures show, the percentage of fibrin does not diminish during fasting, and fasting cats can re-form the fibrin as readily as normal cats.

Number of Experiment.	Hours of Fasting.	Number of Defibrination.	Date.	Weight of Animal.	Total Fibrin in grams.
				grams	
XVI	48	1st	Feb. 12	2.647	0.5816
	72	2d	" 13	0.1600
	120	3d	" 15	2.457	0.4355
	192	4th	" 18	2.245	0.7405
XVII	51	1st	" 12	2.459	0.2425
	125	2d	" 15	2.275	0.5346
XVIII	72	1st	" 13	3.817	0.4844
XIX	30	1st	" 13	2.783	0.5533
XX	78	1st	" 15	2.496	0.4040
	144	2d	" 18	2.320	0.3757
	240	3d	" 22	2.046	0.2257
XXI	96	1st	" 16	3.077	0.1900
	168	2d	" 19	2.842	0.5178
	216	3d	" 23	2.564	0.2450

Attention is invited particularly to Experiment XVI, in which more fibrin was obtained on the fourth defibrination after 192 hours of fasting than on the first determination. In Experiment XVII after 125 hours of fasting the amount of fibrinogen was more than trebled, and no diminution in the power of forming fibrinogen below that of normal cats is to be remarked in any case. The conclusion is justified that a supply of proteid food is not necessary for the formation of fibrinogen. In other words, the transformation of the proteids in the diet into fibrinogen by the mucous epithelium would seem not to be an essential source of the fibrinogen of the blood.

If fibrinogen is not simply transformed food proteid, but is formed from some tissue in the wall of the intestine, it is probably formed either by the lymphatic tissue or by the mucous epithelium. To decide between these possibilities is by no means easy. I have endeavored to decide the matter indirectly by a study of the relation of the fibrinogen content of the blood to the leucocytes.

The relation between the number of leucocytes in the blood and the per cent of fibrinogen. — Pfeiffer,¹ in a recent paper, has endeavored to show that a constant relation exists between the number of leucocytes in the blood and the percentage of fibrinogen. Pfeiffer has here fallen into the error which Horbaczewski made in endeavoring to establish a similar relationship for uric acid. As the following figures show for fibrinogen, and as Weintraud, Klempner, and many others have shown for uric acid, the relationship is between the extent of the decomposition of the leucocytes and the excretion of uric acid and the percentage of fibrinogen. When the number of leucocytes are increased in the blood, it does not necessarily follow that an increase in decomposition of the leucocytes has taken place. Hence one often finds a large excretion of uric acid, and a heavy fibrin per cent coexisting with a small number of leucocytes and *vice versa*. The following figures speak for themselves. The leucocytes were counted in the blood of the femoral or carotid arteries, with the exception of Experiment XXXII, in which the blood was taken from the vena cava.

These figures show that there is no relationship between the number of leucocytes in the carotid blood and the percentage of fibrin. In many cases of abnormally large numbers of leucocytes the fibrin percentage was also increased; in other cases, however, the direct

¹ PFEIFFER: Zeitschrift für klinische Medicin, 1893, xxiii, p. 215; Centralblatt für innere Medicin, 1899, No. 1.

Experiment.	Weight of Cat in grams.	Number of Defibrination.	Leucocytes per cubic millimetre.	Per cent of Fibrin.
XI	3,651	3d	46,400	0.19
XI	3,686	4th	28,480	0.22
XI	3,250	5th	69,920	0.54
XIII	2,754	1st	12,000	0.28
XIII	2,799	2d	19,360	0.15
XIII	2,152	3d	41,920	0.96
XV	3,272	1st	27,200	0.19
XX	2,046	5d	16,000	0.17
XXI	2,842	2d	17,600	0.26
XXI	2,564	3d	37,280	0.15
XXII	3,130	1st	14,400	0.15
XXV	2,229	1st	13,120	0.28
XXVI	3,147	1st	17,280	0.30
XXVII	2,580	1st	35,200	0.53
XXVIII	3,230	1st	24,000	0.23
XXIX	3,052	1st	23,200	0.27
XXIX	2d	40,000	0.35
XXX	2,879	1st	33,344	0.29
XXXI	3,223	1st	19,200	0.13
XXXII	1,445	1st	22,880	0.95
XXXIII	2,800	1st	64,480	0.175
XXXIV	2,000	1st	28,920	0.24

reverse was the case. If the abnormally large number of leucocytes persists for any length of time, as in the following suppuration experiments, however, the fibrin content is abnormally increased.

The influence of suppuration on the per cent of fibrinogen.— Since the days of venesection it has been observed that in conditions of inflammation the blood clots more slowly, the blood corpuscles sink more rapidly, producing the well known *crusta inflammatoria*, or *phlogistica*, and the quantity of fibrinogen is increased. Hammar-

sten¹ states that the per cent of fibrin is increased in pneumonia, erysipelas, rheumatism, and suppurative processes. He gives, however, no comparative figures. Pfeiffer² has recently re-examined the matter and confirmed the older observers. The quantity of fibrinogen is reported to be unaltered in uraemia. It is difficult to find exact determinations upon which these conclusions are based. It is a common observation of physiologists that blood drawn from animals having suppurating wounds appears to yield more fibrin on defibrination than normal blood.

To obtain more exact information on this point, several animals suffering from suppurating eyes and old wounds were examined. Suppuration was induced artificially in others by means of setons through the neck muscles, by croton oil aided by a tar plaster, or by allowing the femoral wounds from previous defibrinations to heal without antiseptic treatment. Sixteen observations were made, the percentage of fibrin being determined directly by Hoppe-Seyler's method, or by computing the total blood as one third of the animal's weight and estimating the percentage from the total fibrin recovered on successive defibrinations. The latter method gives results somewhat too low, so that the percentage should be higher than that indicated. The result shows conclusively that the suppuration, if continued for two to three days or longer, increases the percentage of fibrin, — in some cases quadrupling it.

Suppuration in any part of the body, however produced, leads to a pronounced increase in the fibrinogen content of the blood. This increase is the greater the more prolonged the suppuration. (Experiments XXII and XIII.) This is the conclusion already reached by Horbaczewski and many others in regard to uric acid excretion. Fibrinogen and uric acid run thus a parallel course, both rising in pneumonia, erysipelas, rheumatism, and suppurative processes. From the chemical nature of uric acid and the observations of Meves, Horbaczewski, Kossel, Minkowski, and many others, there is little doubt that uric acid is derived chiefly from the chromatin of the white blood corpuscles set free on their disintegration. Uric acid, aside from that derived from the nucleins of the food, may thus be considered a measure of the decomposition of the leucocytes. The close

¹ HAMMARSTEN: *Lehrbuch der physiologischen Chemie*, dritte Auflage, 1895, p. 152.

² PFEIFFER: *Zeitschrift für klinische Medicin*, 1893, xxiii, p. 215; *Centralblatt für innere Medicin*, 1899, No. 1.

Experiment.	Per cent of Fibrin.	Area Suppurating.
XI (c)	0.54	Neck.
XII (b)	0.38	Femoral wound.
XVI (c)	0.29	" "
XVI (d)	0.49	" "
XVII (b)	0.47	" "
XIX (b)	0.55	" "
XXI (b)	0.27	" " (slight).
XXI (c)	0.14	" " "
XXII (a)	0.17	Back (plaster).
XXII (b)	0.33	" "
XXII (c)	0.51	" "
XXII (d)	0.61	" "
XIII (a)	0.28	Neck (seton).
XIII (b)	0.15	" "
XIII (c)	0.96	" "
XXXII (a)	0.95	Eyes.
Average per cent of fibrin in suppurating cats, 0.41 . Maximum, 0.95; minimum, 0.15.		
Average per cent of fibrin in ten normal cats, 0.20 . Maximum, 0.26; minimum, 0.14.		

parallelism between the uric acid excretion and the fibrinogen content of the blood indicates that the two have a common source. The increase of fibrinogen in all cases of prolonged leucocytosis, in all suppurations or inflammatory conditions, points with the other evidence gained towards the leucocyte as the origin of that substance, rather than to the intestinal epithelium. I believe we may take the fibrinogen content of the blood as an accurate measure of the extent of the leucocytic decomposition.

Fibrinogen being derived from the leucocyte, what part of the body of the leucocyte does the fibrinogen represent?

Fibrinogen presents none of the characteristics of the nucleo-

proteids, hence it would not seem to be derived from the nuclei of the white blood cells. In the cytoplasm of the cells it will be remembered that the reticulum often takes a fibrillar form, these fibrils strongly reminding one of the fibrin fibrils. Fibrinogen has not thus far been isolated from the lymph cells or leucocytes, and hence whether the fibrinogen corresponds to the fibrillar substance in the cytoplasm of the leucocyte must be left temporarily in abeyance. The theory of such a correspondence is, however, an enticing one.

THE RETARDATION OF BLOOD CLOTTING AFTER CUTTING OFF THE CIRCULATION FROM THE INTESTINE

In the course of the experiments in which the intestinal area was cut out of the circulation, observations which are not without interest were made on the effects of the operation on the clotting of the blood. As first observed by Stolnikow¹ and confirmed by Pawlow,² if the blood be confined to a circulation through the heart and lungs, it rapidly loses its power of clotting. Bohr³ some time later found that if the intestinal area was cut out of the circulation the time of clotting was greatly prolonged. He did not determine to the absence of what parts of the intestinal area the loss of the clotting power of the blood was due. The opinion has been expressed that this loss of power of clotting is due to the disappearance of the fibrinogen. Since Bohr's work both Lilienfeld⁴ and Contjean⁵ have repeated his experiments, but failed to confirm his results. My own experiments fully confirm Bohr, and show also what part of the area is necessary that clotting may persist.

Experiment XLIII. Cat. Female. Fasting twenty hours. At 10.30 A.M. I ligatured the coeliac axis and superior mesenteric arteries. Before ligaturing, the blood yielded its fibrin on two minutes whipping. The carotid blood contained 0.214 per cent of fibrin. At 2.40 P.M., four hours afterward, a second sample of blood was drawn from the carotid. It clotted with extraordinary slowness, the fibrin not beginning to form until the blood had been whipped ten minutes. It continued to form slowly for the next thirty minutes. The blood contained, however, as much fibrin as before, *i. e.* 0.213 per cent.

¹ STOLNIKOW : Archiv für Physiologie, 1886, pp. 1-66.

² PAWLOW : Archiv für Physiologie, 1887, p. 452.

³ BOHR : Centralblatt für Physiologie, 1888, p. 261.

⁴ LILIENFELD : Archiv für Physiologie, 1892, p. 150.

⁵ CONTJEAN : Archives de physiologie, 1895, p. 245.

This experiment not only confirms Bohr, but it shows that the retardation of clotting is not due to any lack of fibrinogen. This is also seen in the following experiments:—

Experiment XLVII. Cat. Male. Before ligaturing, the carotid blood contained 0.231 per cent of fibrin yielded on a few moments' whipping. At 10.15 I tied the superior and inferior mesenterics, the coeliac axis, and the mesenteric vein. At 3.15, five hours later, blood drawn from the carotid did not begin to coagulate for thirty minutes, and on removal of the first clots fibrin kept on forming for several hours. This blood yielded 0.259 per cent of fibrin.

Experiment LVII. Cat. Female. In digestion. Before operating, the carotid blood yielded 0.160 per cent of fibrin. At 10.15 the superior mesenteric, the coeliac axis, and the portal and hepatic veins were ligatured. At 4 P.M. the cat died; 10.4867 grams of blood drawn from the inferior vena cava at once thereafter yielded 0.157 per cent of fibrin. This blood did not begin to yield fibrin until it had been whipped thirty minutes, and as in the previous experiments the fibrin kept on forming for several hours.

These experiments show that the loss of clotting power is not due to a lack of fibrinogen. The blood clots as if it were very poor in fibrin ferment, since, as shown by Hammarsten, Schmidt, and others, solutions of fibrinogen poor in ferment clot slowly, and the clotting lasts several hours.

In the foregoing experiments the circulation was cut off from the whole intestinal area. The experiment on Cat LVIII, in which both the spleen and pancreas were removed and in which no diminution in clotting power resulted, shows that it is not to the lack of these organs that the retarded clotting is due. The loss of clotting power must hence be due to cutting the circulation off from the intestine or liver. The following experiment shows that it is a consequence of cutting off the circulation from the intestine:—

Experiment XLVIII. Cat. Female. Ligatured superior and inferior mesenteric arteries and mesenteric vein. The circulation persists through the liver, pancreas, spleen, and stomach. Before ligaturing, the carotid blood contained 0.261 per cent of fibrin. One hour later a portion of blood was drawn from the carotid. The corpuscles settled to the bottom; clotting in the clear plasma began in thirty-five minutes. The blood yielded 0.247 per cent of fibrin.

We may hence conclude that after cutting off the circulation from the intestine the blood largely loses its power of clotting. This is due to the absence of the intestine, and is not affected by the presence or absence of the spleen, pancreas, or liver. The retardation of the

clotting is not due to a diminution in the amount of fibrinogen in the blood, but probably to a diminution of the fibrin ferment.

THE ANALOGY BETWEEN KARYOKINESIS AND BLOOD CLOTTING.

The clotting of the blood has hitherto generally been regarded as an important and striking property of that fluid, comparable indeed to the clotting of milk and muscle plasma, but without analogy elsewhere. For although the blood has long been regarded as a living tissue, no process analogous to the clotting of the blood has been thus far recognized in other living tissues. Schmidt, for example, in the introduction to his treatise on the blood emphasizes the fact that the clotting of blood when shed and its remaining fluid in the body are vital processes, and Lilienfeld sums up his observations on the origin of fibrin ferment with the statement that clotting is a function of the cell nucleus. But neither Schmidt nor Lilienfeld has recognized any similar process taking place in the living cell, a fact the more remarkable since fibrin ferment was found by Schmidt to be a universal component of nucleated protoplasm. It is, however, from the cellular point of view that the clotting of blood is of especial interest, for however important in stopping hemorrhage this property may be, it is of vastly greater importance if it typify one of the fundamental properties of the cell; for in the latter case it renders that property accessible of study by chemical methods, and the brilliant results already obtained by Schmidt,¹ Hammarsten, Lilienfeld,² and many others in blood clotting may be applied directly to the corresponding cellular process, throwing thereby much light upon it.

I have elsewhere³ briefly indicated the remarkable analogy between blood clotting and indirect cellular division. The present study of the origin of fibrinogen was undertaken largely with the hope of obtaining a better insight into this relationship. The parallelism between the two processes is a striking one. Fibrin ferment is found in all nucleated cells. It is not found in nuclear-free protoplasm, such as the red blood corpuscles. It has been obtained from the spermatozoa, leucocytes, thymus gland, liver, kidney, muscle, many moulds, bacteria, and plants. Its general presence in such widely different cells points towards its connection with some common cellular function. The varying richness of different cells in the ferment is ex-

¹ SCHMIDT: *Zur Blutlehre*, Leipzig, 1892, p. 1.

² LILIENFELD: *Archiv für Physiologie*, 1894, p. 555.

³ MATHEWS: *New York medical journal*, 1898, lxviii, p. 515.

tremely suggestive. The spermatozoa yield it in the largest amounts, then come the leucocytes, certain moulds, the thymus gland, and tissues prone to division. The weakest solutions are obtained from the brain, liver, and pancreas. The fact that the ferment is derived only from nucleated cells indicates its origin in the nucleus, and the chemical and microscopical studies of Lilienfeld, Pekelharig, Halliburton, Schmidt, and others have led to the general conclusion that fibrin ferment itself is either a nucleo-proteid (chromatin) or a derivative of chromatin. Fibrin ferment exists in the cells in an inactive form, and is set free by the action of calcium salts, lecithin, and other substances called by Schmidt zymoplastic. In blood clotting, therefore, a substance comes from the nucleus, acts on the fibrinogen in solution in the blood, throws it into the form of fibrils, which, after forming, contract.

The resemblance of this process to cellular division is of great interest.

In karyokinesis a substance, the centrosome, becomes active. This substance takes its origin in the nucleus or its immediate vicinity. The substance of the centrosome acts upon the cell body, throwing a substance there into the form of fibrils which penetrate the cell in all directions. These fibrils after formation contract like the fibrin fibrils. These resemblances may be tabulated as follows:—

<i>Clotting.</i>	<i>Karyokinesis.</i>
(a) Induced by fibrin ferment.	(a) Induced by centrosome.
(b) Fibrin ferment takes its origin in the chromatin.	(b) The centrosome arises in the nucleus or its vicinity.
(c) Fibrin ferment exists normally in all cells in an inactive form.	(c) The centrosome exists in many cells in an inactive form.
(d) It is present in largest amounts in the spermatozoa.	(d) The spermatozoa induce cell division.
(e) The inactive form of the ferment is changed by calcium salts and lecithin to the active form.	(e) Without calcium, many cells cease to divide. Lecithin stimulates cell division. Eggs are particularly rich in lecithin.
(f) The fibrin ferment acts on fibrinogen and throws it into fine fibrils.	(f) The centrosome acts on the cytoplasm and throws it into fibrils.
(g) These fibrils penetrate the blood.	(g) Fibrils penetrate the cell.
(h) After formation the fibrils contract.	(h) After formation the fibrils contract.
(i) If left in the blood the fibrils disappear. ¹	(i) In most cells the fibrils later disappear.
(j) The fibrinogen of the blood is derived from the leucocytes on disintegration of the latter.	(j) The cytoplasm of the leucocyte contains a substance capable of being thrown into a fibrillar form.

¹ DASTRE, A. : Archives de physiologie, 1893, xxv, p. 661.

This parallelism is so close, the optical features are so similar, the present investigation so clearly indicates the origin of the fibrinogen in the leucocyte, and previous observations have shown such a remarkable similarity of origin for the centrosome and fibrin ferment, that I am inclined to suspect that karyokinesis and the clotting of blood are one and the same process; that the causes of clotting are identical with the causes of division; that the centrosome is fibrin ferment; that the fibrils of the karyokinetic figure are composed of fibrin. The proof of this hypothesis is, however, lacking, and can only be furnished by the isolation of fibrinogen from cells.

SUMMARY OF OBSERVATIONS.

The fibrinogen may be removed from the blood of cats by repeated bleedings, defibrinations, and reinjections.

The operation or the lack of the fibrinogen appears to cause no serious or characteristic symptoms.

After defibrination the fibrinogen is rapidly re-formed, and in two to three days is present in normal or more than normal amounts.

The re-formation of the fibrinogen takes place normally in the absence of the spleen, the pancreas, the kidneys, the reproductive organs, or the brain. Hence these organs cannot be important makers of fibrinogen.

The fibrinogen is not re-formed, or is re-formed at a greatly reduced rate, if the small and large intestines be removed.

The paraglobulin of the blood is not converted into fibrinogen outside the body, or by the vascular endothelium, the skeletal muscles, skin, or other tissues of the leg.

In prolonged perfusions of the leg with defibrinated blood no fibrinogen is added to the blood. Hence fibrinogen is not formed in this region of the body.

The blood of the inferior vena cava both below and above the kidney is poorer in fibrinogen than the carotid blood.

The blood of the mesenteric vein is constantly somewhat richer in fibrinogen than the arterial blood.

Fibrinogen is not derived directly from the proteid constituents of the food, since it is readily re-formed after six to ten days' fasting. The fibrinogen content of the blood does not diminish during fasting.

There is no direct relationship between the number of leucocytes in the blood at a given time and the per cent of fibrinogen. A small

number of leucocytes may coincide with a heavy fibrinogen per cent, and vice versa.

If leucocytosis be prolonged for several days by suppurations induced by setons, plasters, local infections, etc., the fibrinogen content of the blood increases. In this respect fibrinogen runs a parallel course with the uric acid excretion.

If the intestinal area be excluded from the circulation the clotting of the blood is greatly retarded. This is due to the lack of the intestinal circulation and not to the absence of the liver, spleen, or pancreas. The slowness of clotting is not due, in such cases, to a lack of fibrinogen, since this remains practically unaltered.

The general features of blood clotting bear a striking resemblance to those of indirect cellular division, or karyokinesis.

CONCLUSIONS.

The facts presented in the foregoing pages clearly point to the decomposing leucocytes of the blood, and chiefly those of the intestinal area, as being the source of the fibrinogen of the blood. This conclusion is supported (1) by the increase in the per cent of fibrinogen in all cases of prolonged leucocytosis accompanying suppuration; (2) by the increase in fibrinogen during leucocythemia; (3) by the increase in fibrinogen in pneumonia, erysipelas, acute rheumatism, peritonitis, and similar inflammatory conditions; (4) by the fact that fibrinogen is not simply transformed proteid of the food, as indicated by its continued formation during fasting and its failure to increase during proteid digestion; (5) by the observation that neither the spleen, muscles, kidney, pancreas, or brain appear to be essential to its formation; (6) by the well known fact that there is present in the cell body of the leucocyte a substance which, by the action of a substance coming from the nucleus or arising in its neighborhood, is thrown into a fibrillar form closely resembling fibrin fibrils, and like them contractile; (7) by the fact that the leucocytes are constantly going to pieces in the body, hence must be adding constantly to the proteid constituents of the blood; (8) by the close correspondence existing between the fibrinogen content of the blood and the excretion of uric acid; and (9) by the fact that the intestine, which is rich in leucocytes, appears to be the chief source of the fibrinogen of the body.

In all diseased conditions in which the uric acid excretion is increased, the fibrinogen content of the blood increases also. Since

it has been shown by Meves, Horbaczewski, v. Minkowski, and others that uric acid is derived in all probability in such cases from a decomposition of the leucocytes, fibrinogen, which runs a parallel course to it, probably has a similar origin. I believe we may take the fibrinogen content of the blood as a true measure of the amount of leucocytic decomposition in the body, a measure far more reliable than the amount of uric acid excreted, since the latter substance is derived also from the nucleins of the food and is liable to be oxydized in part to urea. The use of the fibrinogen content of the blood as a convenient measure of leucocytic decomposition will possibly yield results of considerable interest in the future.

It is clear that if fibrinogen is being constantly set free in the blood it must be constantly consumed, and the questions arise: By what organs is it consumed? is it a necessary food? is not its nutritive function the chief reason of its being? and what are the results of its removal from the body? These questions have not as yet been answered. It would be interesting to know, also, whether any of the symptoms of rheumatism, pneumonia, or other diseases, such as the rapid wasting of the tissues or the high temperature, are a consequence of its presence in the blood in abnormal amounts.

Finally, if fibrinogen is derived from the leucocytes, as the preceding considerations indicate, and if Schmidt's and Mörner's observations on paraglobulin indicating its origin in the leucocyte prove well founded, the conclusion would seem obvious that the proteids of the blood are derived directly from the leucocytes. This would strongly confirm Hoffmeister's view that the leucocytes are pre-eminently active in proteid absorption and assimilation. It would lead to the interesting conclusion that the organism lives on its leucocytes much as the egg-cells of some forms live on their follicle cells.¹ If this were so, it would explain (1) the true function of the leucocytes and the elaborate arrangements for their production in the body; (2) their congregation and great reproduction in the intestinal area during a proteid meal; (3) the positive chemotaxis they exhibit toward the proteids, albumoses, and other products of

¹ This brings us back to the old theory of His, now generally abandoned, according to which the leucocytes are the lineal descendants of the follicle cells. The embryonic origin of the leucocyte may possibly throw an interesting light on the peculiar relation they hold to the organism. One is strongly tempted to homologize them with the follicle cells of *Salpa*, which serve as food for the developing embryo, and with the vitellophags, which absorb the yolk.

digestion; (4) the maintenance of the proteid constituents of the blood during fasting; (5) the fate of the bodies of the leucocytes when they disintegrate; (6) the fact that no products of digested proteids are found in the blood during proteid digestion. It would make the leucocyte, in fact, a storehouse of the surplus proteid food of the body, just as the liver cell is a storehouse of surplus carbohydrate food.

Enticing as this idea is, it cannot be finally accepted until serum albumin, paraglobulin, and fibrinogen have been isolated from the leucocytes. In the absence of this evidence the rôle of the leucocyte sketched above, although supported by indirect evidence worthy of consideration, must be considered a provisional hypothesis as yet lacking a demonstration.

A CONTRIBUTION TO THE COMPARATIVE PHYSIOLOGY OF COMPENSATORY MOTIONS.

By E. P. LYON.

[From the Hull Physiological Laboratory of the University of Chicago.]

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INTRODUCTION.

WHEN a frog is placed upon a horizontal turntable and revolved not too rapidly, the head of the animal turns to one side in the direction opposite to that of revolution. This is an example of compensatory motion. The animal is said to compensate or tend to compensate the passive revolution by trying to keep its head in its original position in space. The head is held in its side-inclined position as long as the revolution is continued. If the turntable is suddenly stopped, the frog moves its head back not only to the normal mid-line of the trunk but beyond, the inclination being now in the direction of the previous revolution. This is an example of "after-effect." After a few seconds the head returns to its normal position.

Pigeons upon being similarly revolved exhibit a bending of the head like that shown by the frog. This motion may amount to 120° , and has been called by Breuer the "reaction movement." Unlike the frog, however, the pigeon does not retain the new position, but with a quick jerk the head is moved suddenly, in the direction of the turntable revolution, part way back to its normal position. This jerk is the "nystagmus phase." Immediately the head begins to move again oppositely to the direction of body revolution, not with a jerk, however, but with slow, steady motion. This is the "reaction phase." After attaining again nearly or quite the maximum angle of the reaction movement, the head

is once more jerked back, and so on, each reaction phase being followed by a nystagmus phase. The result is a series of pendulous motions in a horizontal plane, slow in the direction opposite to that of general revolution, quick in the direction of revolution. The slow reaction movements are compensatory. The nystagmus is a convulsive reflex of secondary character.

If the revolution be long continued, the vibratory motions as a rule become weaker and finally cease. When now the turntable is stopped, the pigeon, like the frog, turns its head in the direction of revolution past the sagittal plane of the body. But unlike the frog, the pigeon's head shows a marked after-nystagmus.

If the head of a pigeon be held during revolution, and in some pigeons regularly without holding, compensation is effected by the eyes instead of by the head. The eyes exhibit the reaction and nystagmus phases in exactly the same manner as does the head. After nystagmus in the opposite direction follows a sudden stoppage of revolution. Rabbits and many other mammals show the same phenomena. The dog-fish (*Mustelus canis*) also compensates rotation about a dorsiventral axis by moving its eyes in the opposite direction. But this motion is accompanied by little or no nystagmus. The eyes are kept in their compensating positions as long as rotation continues. The eyes, furthermore, sometimes show the same motions when the animal moves voluntarily and normally. A dog-fish, for example, when swimming on its side may keep the upper eye to the ventral, the lower one to the dorsal side of the orbit. Compensatory motions are not, therefore, confined to passive rotation by external means.

Mice and many other vertebrates when revolved not too rapidly upon a horizontal turntable run in circles opposite to the direction of revolution. If the turntable moves clockwise, the animals go the reverse. Here compensation is effected by locomotion.

The compensatory motions so far mentioned, whether of the head, eyes, or entire body, tend to keep the same field of vision before the eyes. In few, if any cases, however, do they accomplish this. Even a slow and small rotation, so far as my measurements show, is almost never entirely compensated. If compensation is a device for retaining the field of vision, it does not perfectly accomplish its purpose.

Besides these compensatory motions certain vertebrates upon passive rotation display compensatory motions which seem intended

to return the body to its original position in space. When the dog-fish, for example, is rotated about a longitudinal axis, the fins move in the same direction as the rotation, not opposite to it as do the eyes.

When the animal is rotated in horizontal planes the compensating positions of head, eyes, body, or appendages are retained, or the motions of the same continued only during the rotation. But when the rotation is in vertical planes,—that is, in vertebrates whose chief axis is ordinarily horizontal, rotation about either a longitudinal or a transverse axis,—the compensating positions are retained as long as the animal is held in an unusual position. It is to be noticed that in horizontal rotations the orientation of the animal with respect to the direction of gravity remains unchanged. In rotation in vertical planes the orientation with respect to gravity is changed. In the former case the compensating positions are not retained. In the latter case they are.

The instances of compensatory motions mentioned above are from the works of Breuer, Ewald, v. Cyon, Lee, and others, and are to be considered types of one general phenomenon.

THEORETICAL CONSIDERATIONS.

For the explanation of these motions on the basis of ordinary reflexes three things have seemed to be necessary: (1) a peripheral organ capable of stimulation by gravity, centrifugal force, inertia, or the stresses and pressures resulting therefrom. This organ should be connected by means of nerves with (2) a centre in the brain which should interpret the stimuli received by it from the peripheral organ, and in turn should be connected by means of nerves with (3) the muscles by which the compensatory motion is brought about. The hypothetical peripheral organ or organs have been the *ignis fatuus* of a generation of physiologists. Not content with simple reflexes, they have originated two new senses, the dynamical and statical, and have devised a very complicated theory for their stimulation. So far as facts go, it is pretty generally admitted that cutting of the optic nerves together with sufficient destruction of the inner ear is sufficient to do away with compensatory motions. As to the theory it may be stated in general terms as the view of many physiologists (1) that the semicircular canals are of a "dynamic" character, being stimulated by the active or passive motions of an animal and arousing compensatory motions which continue only during stimulation of the nerves ending in these canals; (2) that the otocyst is a

"static" organ, constantly stimulated by the pull of the stone upon nerve endings and leading to the continuance of compensating positions whenever the body is out of its normal orientation with respect to the vertical. While recognizing the beauty of this theory, and the array of experimental evidence which has been brought forward in its support, the writer has come to doubt the validity of the theory so far as compensatory motions are concerned.

The semicircular canal theory rests first upon the anatomical fact that the canals are arranged in three planes of space approximately at right angles with each other. They seem therefore by this geometrical relation to be organs well adapted to the mediation of movements either in the plane of any one canal or, on the principle of the parallelogram of motions, in any other plane whatsoever. Is this arrangement of canals able to explain all compensatory motions? Professor Loeb calls my attention to the following argument: Suppose a frog is placed upon a horizontal whirling table, so that it faces the periphery of the wheel. In the accompanying figure (Fig. 1), *C* represents the axis of rotation and *A* the position of the animal's head and the horizontal canals. The table is then rotated clockwise or in the direction of the arrow *D*. The animal turns its head to the left in the direction of the arrow *C*. According to the theory, there is a flow of endolymph from the right canal into its ampulla, or at any rate an increase of pressure in the latter and a flow away from or decrease of pressure in the left ampulla. This difference in liquid pressure or flow acts as a stimulus which arouses the proper chain of internal processes leading to the contraction of muscles and the visible bending of the head. Briefly stated, the theory would assert that stimulation of the right ampulla leads to a bending of the head to the left.

But suppose the animal is so placed upon the turntable as to face the axis. This position is marked *B* in the diagram. Let the turntable be revolved as before. The head bends as before *to the animal's left*, in the direction of the arrow *C'*. According to theory there should be increase of pressure in the ampulla of the side moving in advance, that is the *left*, and decrease of pressure in the *right*, exactly the reverse of the condition when the animal faces outward. *But the same reaction follows.* The animal bends its head to its left.

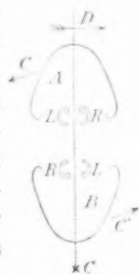


FIGURE 1.

According to theory, stimulation of the left ampulla should cause the head to be inclined to the right. It does not do so. Therefore the theory is incorrect.

The adherents of the semicircular canal theory were forced to admit its insufficiency in cases where compensatory positions were held after rotation had ceased. They fell back upon the otolith and the "static equilibrium." It seems to me the case cited demonstrates the inability of the theory to account for certain "dynamical" phenomena as well. Cyon and Ewald long ago showed that the canals could be compressed or plugged up without interfering with compensatory motions.

ROTATION EXPERIMENTS.

The types of compensatory motions which I mentioned in the opening of the paper were all found first in vertebrates, animals having semicircular canals. If compensatory motions were confined to this group of animals at least the argument of coincidence would be favorable to the theory. But they are not so confined. Furthermore, if the reactions were found only in animals having otoliths or otocysts, we might with propriety seek a relation of cause and effect. But they are found in animals, having neither semicircular canals nor otocysts. I shall show that every type of compensatory motion found in vertebrates is duplicated in invertebrates; that even the accompanying reflex nystagmus is discernible, demonstrating the unity of the phenomena in both divisions of the animal kingdom; that these reactions occur not only in animals with semicircular canals, but also in those destitute of otocysts; and that therefore the canal-otocyst hypothesis is either entirely incorrect or of merely secondary importance.

Kreidl,¹ in his famous experiment in which he caused *Palaemon* on moulting to replace its otoliths with metallic iron, seems to have been the first to notice compensating motions of the invertebrate eye. "It is interesting to note," he says, "that in these invertebrate animals also the reflex movement of the eye is present." This seems to have been observed in sidewise inclination only. He found that this crustacean reacts upon the turntable exactly like some vertebrates, running in circles opposite to the direction of rotation.

The animals with which Kreidl experimented are provided with

¹ KREIDL: Sitz-Ber. der kais. Akad. der Wissensch., Wien, 1893, cli, Abth. 3, p. 149.

otoliths. Is it possible that compensatory motions can be present in crustaceans without otoliths? Indeed it is, for Clark¹ found that *Gelasimus* and *Platyonichus* give very prompt responses upon rotation in vertical planes. And these responses agree point for point with those manifested, for example, by the dogfish, which possesses semicircular canals and otoliths. For instance, on rotating the crabs to the right about a longitudinal (horizontal) axis, the eyes roll to the left, and their new positions are retained as long as the animal is held in an inclined position. Yet Clark's results were looked upon not as contradictory, but confirmatory of the commonly accepted hypothesis.

Gelasimus was revolved on a horizontal turntable and "showed no reaction to slow turning," and when rapidly revolved "no after-effects were observed." Upon turning the crab about its dorsi-ventral (vertical) axis, no compensating movements of the eyestalks were discovered. This, of course, was favorable to the theory, as the animal has no semicircular canals.

After the appearance of Clark's paper, Professor Loeb desired me to perform similar experiments on the crayfish at Wood's Holl. This desire was stimulated by the recollection of certain experiments of his own upon the pelagic gastropod *Pterotrachea*, the results of which were never published. This form is very transparent, and the otocyst, which lies some distance from the central ganglia, can be seen plainly and easily taken out with a hooked needle. Its removal was accompanied by no disturbance of the equilibrium whatsoever, although the animal is very active. It therefore seemed to Professor Loeb that further investigations were necessary before the theory of the equilibrium function of the otocyst should be given universal application or acceptance. This seemed to him the more needful from the fact that insects and other forms possessing no otocysts are yet able to preserve their equilibrium perfectly.

The crayfish.— In order to detect and measure the movements of the eyestalks upon rotating the crayfish around a longitudinal axis, the apparatus shown in Figure 2 was devised. It will be referred to as Apparatus A.

To the base (*a*) are fastened the uprights which carry the rotating board (*b*). This board can be turned by the crank (*f*) into any desired position and held there by the spring-brake (*g*). The amount of turning of the board and consequently of the animal

¹ CLARK: Journal of physiology, 1896, xix, p. 327.

is read from the graduated circle upon the upright (*c*). The animal is fastened to the rotating board by means of rubber bands. The

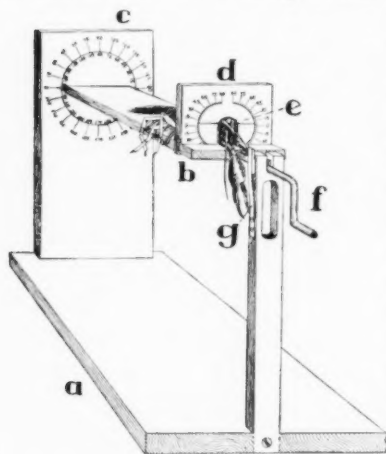


FIGURE 2.

block (*d*) is notched so as to fit over the animal just back of the eyes, and is fastened in this position by a rubber band extending under *b*, as shown in the figure. To this upright block is attached the mirror (*e*) carrying arcs of graduated circles whose centres are at the bases of the eyestalks, and therefore in the axis of rotation of the latter. It was found that light indicators could be glued to the eyestalks without interfering with their motions. Bristles were generally used for this purpose. These indicators extended before the mirror and slightly beyond the edge of the

graduated circles. By this device errors of parallax were avoided, and motions of the eyes of one degree or less could be detected.

In the following experiments the words "left" and "right" will refer to the animal's left and right.

Rotation in a transverse plane, i. e. about a longitudinal axis.—The turning of the eyestalks of the crayfish upon rotation of the animal about a longitudinal axis has been mentioned by Clark. He did not, however, make a study of it. Just as in other crustaceans studied by him and in various vertebrates described by several observers, the eyes of the crayfish tend to keep their positions in space when the animal is turned in transverse planes. This tendency leads to a movement of the eyes (with reference to a point on the animal) opposite in direction to the motion of the animal itself.

If the animal is stopped at any point in an arc of 180° from its normal horizontal position, the eyes retain the position into which they have come during the rotation. The details of the reaction will now be described.

Normal animals were placed in Apparatus A. So long as the animals remained in their usual horizontal position, the eyestalks were held at an angle of 18° to 25° above the horizontal. Turning

an animal towards its right up to nearly 90° is accompanied by an approximately proportional movement of the eyes towards the animal's left. This movement of the eyes, although proportional to the amount of rotation, is at no part of the arc equal to it. In other words, the eyes do not keep their positions in space but only "tend" to do so. The two eyes do not move through equal arcs. Rotation through 90° towards the animal's right is accompanied by a movement of the right eye towards the left (or dorsally) of about 35° , and of the left eye towards the left (or ventrally) of about 25° .

If the rotation be continued beyond 90° , the movement of the eye-stalks continues. It is, however, no longer proportional to that of the animal, but becomes less and less for each increment of rotation. After rotation towards the right through 120° the left eyestalk becomes stationary at about -10° on the mirror scale, having moved about 30° . Continuing the rotation to 150° the right eye also becomes stationary at about 60° to 65° , having moved in all over 40° .

The eyes make no further movements until the animal has been turned through about 200° from its original horizontal position, when they begin to move in the same direction as the animal, in other words, back towards their normal position. The movement is irregular and may continue after the animal has come to rest in any position between 200° and 300° , but the eyes never come back to the position they would have occupied had the animal been rotated in the opposite direction to the same point.

At about 320° the eyestalks again begin to turn in a direction opposite to that of the animal; and at 360° , the animal having made a complete rotation, the right eye is a few degrees above, and the left eye a few degrees below, the normal position.

These movements are represented graphically in Fig. 3. The abscissas show degrees of rotation of the animal towards the right. The ordinates show the position of the eyes on the mirror scale. The continuous curve represents the motion of the right eye with reference to the line connecting the bases of the eyes. The dotted line represents the motion of the left eye in similar manner. An upward movement of the locus indicates motion of the eye dorsally; downward movement, motion ventrally.

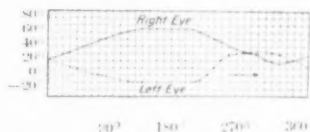


FIGURE 3. The turning of the eyes of crayfish for rotation through 360° to animal's right.

In the case of the right eye, therefore, an upward movement of the locus denotes turning in a direction opposite to that of the animal. In the case of the left eye, the reverse is true. If the animal were rotated towards its left, the motions of the eyes and their resulting loci would be reversed.

The amount of eye motion for a given arc of rotation, say 90° , varied with the quickness of rotation. If the animal was rotated slowly, and especially if it was stopped several times in the arc, the turning of the eye corresponding to 90° of rotation was larger by 5° or more than when the animal was rotated quickly.

Compared with Clark's results on *Gelasimus* the following differences are observed on turning crayfish around an antero-posterior axis: (a) less movement of eyes; (b) continuation of motion of eyes when animal is rotated beyond 90° ; (c) upon rotation beyond 180° the eyes never take the positions they would have had if the animal had been brought by opposite rotation into the same position.

To test crayfish for after-effects, normal animals were quickly rotated ten times about a longitudinal axis. No after-effects were ever observed.

Rotation around a transverse axis. — Just as Clark found in *Gelasimus*, the crayfish upon being turned forward around a transverse axis moves its eyestalks backwards; and, upon being turned backwards, turned its eyes forwards. As there seemed to be nothing particularly different in these motions from those already described, they were not extensively studied.

Rotation about a vertical axis. Nystagmus. — Early in my work I found that, contrary to Clark's assertion for *Gelasimus*, the crayfish clearly showed compensatory motions of the eyes on rotation in horizontal planes about a dorsi-ventral axis. As such a reaction had never been reported, I studied it in some detail, and devised a modification of Apparatus A for that purpose.

The animal was fastened by means of rubber bands to a piece of flat wood. At each side of the head a small piece of board about 1.5 cm. thick was fastened in such a way as to bring small horizontally placed mirrors bearing graduated quadrants of circles a few millimetres below the eyestalks. The bristles fastened to the eyestalks extended over these mirrors. The animal was rotated either by hand or by the kymograph, the holder resting on the top of the drum.

The eyestalks are normally held at angles of 65° to 80° with the median, dorsi-ventral plane. When the animal is rotated around a

vertical axis they move 10° to 18° in the opposite direction. For the first few degrees of rotation the eyes seem to retain their position in space absolutely, *e. g.* if the animal was moved through 5° towards its right, the eyes moved 5° towards the animal's left. In this respect the rotation in a horizontal plane is different from that in a vertical plane, in which the movement of the eyes is for a time proportional to, but at no part of the arc equal to, the angular motion of the animal. Beyond 5° , however, the eyes did not keep pace with the angular motion of the animal.

When the eyes had moved 10° to 18° as indicated, they became stationary, and then with a quick jerk moved 3° to 5° towards their original position. Both eyes moved at the same instant and through the same angle. If the rotation of the animal was continued, these oscillatory motions of the eyes were repeated at fairly regular intervals for some time. After several rotations, however, they became fewer and finally ceased.

Using Breuer's nomenclature, the eyestalks of the crayfish show, therefore, a "reaction movement" through 10° to 18° , a "nystagmus phase" of 3° to 5° .

Upon bringing the animal into a condition of rest no after-effects of any kind were observed.

The nystagmus effects were the same when the animal's field of vision was made to rotate with it by surrounding it with a pasteboard cylinder. I should, however, state that a space was left at the top for observing the animal, and therefore the experiment was perhaps incomplete. After rotating the animal for a time in one direction, reversal of the motion made the nystagmus more frequent and regular.

No facilities were at hand for observing the animal under rapid rotation. Within the limits of velocity in which the eyes were directly observable, the effects were always found. They were most favorably seen when the rotations numbered two or three per minute. Under such circumstances there were seven to ten nystagmus movements per rotation. I was so much surprised and impressed with these results that at Woods Holl last summer I repeated the experiment on Clark's animals, *Gelasimus* and *Platyonichus*, as well as several other crabs and the lobster. In no instance did I fail to find compensatory motions of the eyes upon rotation about a dorso-ventral axis. In *Gelasimus*, the nystagmus is especially marked and frequent. The demonstration needed no apparatus whatsoever, and never failed in dozens of individuals examined.

Experiments with the horizontal turntable. — Like Miss Bunting,¹ I was unable to detect in large crayfish any inclination to rotatory locomotion when they were revolved on a horizontal turning table. On the contrary, Kreidl reports a very prompt reaction in *Palaeon*. And upon experimenting with small crayfish about 2-3 cm. long, I was surprised to find a very lively reaction to the turning table. The animals moved in small circles opposite to the direction of revolution. It was not an effort on the part of the animal to keep as far as possible from the observer. At Wood's Holl I tried *Gelasimus*, and found that these and other crabs show the same reaction. When revolved towards the right, they run in circles to the left just like a mouse or salamander.

Insects. — Dr. Loeb² has called attention to the fact that certain insects experimented upon by him, *e.g.* houseflies and beetles, react to revolution on a horizontal turntable. They behave exactly like vertebrates, running in circles opposite to the motion of the turntable. Here then is a very definite compensatory motion effected by animals not only without semicircular canals but also devoid of otocysts. K. L. Schaefer³ later revolved crabs, earthworms, caterpillars, various insects, and snails, and claimed that only the latter gave any response. Upon further study of the insects, however, I have been able to establish in them almost every type of compensatory motion which has been described in vertebrates. In all the following experiments it will be understood that imagoes were used.

A large beekiller (*Asilus*) was slowly revolved upon a horizontal turntable and showed very prompt reaction of the kind described by Dr. Loeb. When it was rapidly revolved, there was no after-effect. The same reaction upon revolution and the same lack of after-effect was manifested by locusts, grasshoppers, dragonflies, several species of flies, ants both red and black, and several forms of beetles. The phenomena would appear therefore to be very common if not of universal occurrence among adult insects.

The potato beetle reacts to the turntable like the other insects, but shows an interesting pseudo after-effect. If the revolution be sud-

¹ BUNTING, M.: Archiv f. d. ges. Physiol., 1893, liv, p. 531.

² LOEB, J.: Der Heliotropismus der Thiere, etc.; Anhang. Einige weitere Versuche über den Geotropismus der Insekten, Würzburg, Georg Hertz, 1890.

³ SCHAEFER: Naturwissenschaftliche Wochenschrift, Nov. 25, 1891; also Zeitschrift für Psychologie und Physiologie der Sinnesorgane, 1892, iii, 2 and 3.

denly stopped, the animal does not cease its circular motion instantly, but continues for an observable interval to move in the same direction as during revolution, *i. e.* opposite circles. After a moment it starts off in a straight line. If this were a true after-effect it would be in the opposite sense to those after-effects seen in vertebrates, which are always in the same direction as the revolution. In the case of this beetle it must be considered, I think, as a sort of "personal equation," an interval between the cessation of the stimulus and the cessation of muscular response. If the beetle is crawling across the turntable and the table is started with a quick jerk through a partial revolution and immediately stopped, the beetle may be found still moving in a straight line. In an instant, the turntable being now at rest, the beetle makes a partial circle opposite to the previous motion of the turntable and then starts off again in a straight line. Almost a second must elapse between the stimulus, whatever it may be, and the corresponding compensatory motion — much too long a time for the latter to be of any benefit to the animal.

Nystagmus. — The head of the beekiller is connected to the thorax by a very slender "neck" and is therefore extremely mobile. When the animals were held in the fingers and rotated about a dorsi-ventral (vertical) axis, the head made characteristic compensatory motions accompanied by nystagmus. Every phase was as clear as in the pigeon. The slow and steady "reaction phase" and the quick and jerky "nystagmus phase" were beautifully distinct. No apparatus or training is necessary to demonstrate this fact. Dragonflies display the same in less marked degree.

Sustained compensatory positions. — When the beekiller is rotated about a transverse (horizontal) axis, the head makes compensatory motions unaccompanied by nystagmus. For example, when the animal is tipped forwards, anterior end downwards, the head moves dorsally, *i. e.* opposite to the motion of the animal. This reaction also is very marked. The proboscis in the normal horizontal position of the body is directed ventrally at right angles to the body axis. But when the animal has been turned forwards through 90° , the proboscis points almost in the longitudinal axis. When turned head upward the opposite occurs; the head moves ventrally and the proboscis comes to lie close against the thorax. *In both of these cases the compensating position is retained so long as the body is held in its new orientation with respect to the vertical.* No one has found an otocyst in the beekiller.

No well marked compensatory motions were seen upon rotating these animals about a longitudinal axis. A twisting of the head, such as the pigeon exhibits, was perhaps to be expected. But I have made no observations as to whether the bekillers are accustomed normally and voluntarily to make such a motion. If not, it would hardly be expected as a reflex.

OPERATIVE EXPERIMENTS ON THE SEMICIRCULAR CANALS AND OTOLITHS.

I owe to Dr. Loeb the ingenious suggestion that Nature has worked out in the flounder the solution of the semicircular canal problem. As is well known, this fish when hatched, and for a considerable time thereafter, is as symmetrical as any vertebrate and has an eye upon each side of the head. Later it gradually turns upon one side and finally becomes quite asymmetrical. The eye of the lower side comes to the upper side, the change being accompanied by marked alteration of the bones of the head; the two sides become different in color and shape; the mouth also gradually loses more or less of its symmetry.

Dissection shows, however, that no shifting has taken place in the brain or in the ear. The transverse axis of the brain, therefore, is now vertical. The morphologically horizontal (external) canals are also vertical; the morphologically vertical canals lie in planes half way between the horizontal and vertical; the stress relations of the otoliths are entirely changed. It would seem, therefore, that here is an animal especially suitable for testing the supposed dynamical and statical functions of these organs. Furthermore the animals are very hardy. Artificial respiration can easily be maintained for an indefinite period. The semicircular canals, though not so easily accessible as those in the cartilaginous cranium of the dogfish, can be laid bare without undue difficulty or delay and with practically no bleeding. In a fair sized fish they are a centimetre or more in length, and therefore of sufficient size to make the operator sure of what he is doing.

The fish used in these experiments was *Pseudopleurinctes Americanus*, the common "winter flounder" occurring at Wood's Holl. It lies upon the right side; the eyes therefore are both upon the upper, left side. Though a bottom fish, it is quite active. If turned "wrong side up" in the water it immediately recovers its normal position without preliminary contact with solid bodies. *The compensatory motions of the eyes are exactly like those of other fish.* Compensatory positions corresponding to changes of orientation with respect to the

direction of gravity are sustained so long as the body is held in its abnormal position. Morphological relations are entirely subsidiary. If the animal be turned in vertical planes the eyes move in the opposite direction in the same planes. Therefore if the canal theory be true, the morphologically horizontal canals are now mediating motion in vertical planes. Considering the asymmetrical position of the right eye this result seems quite wonderful. It is possible that the muscles of this eye have also so shifted that its present motion, which is opposite of course to its original motion when on the right side of the fish's body, is accomplished by the same muscle or set of muscles as before. On the other hand, if the shifting of the eye is unaccompanied by such rotation, the canal theory would demand a considerable shifting of nerve connections. I have not been able either to determine the matter by dissections or to search the anatomical literature for its possible demonstration.

Throughout my experimental work I have tried to adhere to the following rules:—

1. If stimulation of a sense organ or part of the nervous system calls forth a certain reaction (motion) it is certain that the stimulated organ is connected by nerves with the centre of this motion, but not that the stimulated organ is itself the centre.

2. If through extirpation of an organ a function is eliminated, it is certain that the extirpated organ is connected by nerves with the centre of this function, but not that it is itself the centre.

3. If after extirpation of an organ a function which ordinarily is lost after this extirpation remains *in one case only*, it is certain that the extirpated organ is not the sole centre of this function.

Bearing in mind the third principle in particular I have tried to give attention to the exceptional cases instead of throwing them out altogether, as is too often the custom, and founding conclusions upon the basis of majorities. This method, one can readily see, demands constant care. The exceptional case of a function retained after removal of an organ whose loss ordinarily entails obliteration of that function may mean nothing more than an imperfect operation, the residue of an organ or a partially severed nerve. It is but fair to state that I have borne these considerations in mind, and that if errors have crept in, they are not attributable to want of conscientious care. Post mortem examinations were always held, and were generally witnessed by Dr. Loeb or Dr. Norman. I might also add that I began these experiments a believer in the otolith-canal theory.

Extirpation of canals. — *The flounder.* — The (morphologically) vertical canals anterior and posterior were removed from both sides. The nerve leading to each ampulla was first cut and the canal and ampulla removed together. Immediately after the operation the animal was revolved about a longitudinal axis and showed normal compensatory motions. Yet all the canals which should mediate such motion had been removed. Revolved about a dorsi-ventral (horizontal) axis, there was little or no compensation. Yet the canals in the plane of this motion were still intact. The slightest stimulation of these (morphologically horizontal) canals still gave strong and perfectly definite motion of the eyes in the plane of the canals. This happened in two animals. These animals were very strong and vigorous. The eyes reacted to touch after the operation and little evidence of shock could be detected.

Remember that there cannot be the slightest doubt as to which canals were removed or as to their entire removal. Yet compensatory motions remained in the plane of the extirpated canals and were largely lost in the plane of the remaining canals, while stimulation of the latter still gave rise to the motion of the eyes in the plane of these canals. It appeared certain to those who saw these animals that there could not be any stimulation of the horizontal canals without motion of the eyes. Yet no motion of the eyes followed rotation in the plane of these canals. The conclusion was irresistible that rotation of the animal did not stimulate the horizontal canals. The compensatory motion seen in normal animals on rotation in this plane must arise from some other source.

In one individual all six canals and ampullæ were entirely removed, and yet a large amount of compensatory motion on rotation about a longitudinal axis remained. It was perhaps a little less than normal. At the same time a small amount could be detected on rotation about a dorsi-ventral axis. My notes say: "Is very lively, eyes held far out, not drawn in at all." And this leads to the remark that it was the strongest animals and the cleanest, quickest operations that gave these results. While in many cases one would find after the operation little or no compensatory motion, yet at the same time evidences of severe shock were observable. Often the eyes showed no reaction to touch and appeared sunken in their orbits. Indeed, toward the last I came to know that if the eyes would not react to touch, there was sure to be no response on rotation. This was more common in the dogfish than in the flounder. It was the strongest, least injured

animals which — from the standpoint of the canal theory — gave the worst results!

The dogfish.—Two specimens of *Mustelus canis* from which all the ampullae were entirely removed gave results very like the above. I quote from my notes:—

Case I.—“Small lively dogfish. All ampullae of both ears removed. Animal very lively after operation. Eyes react to touch. Placed in aquarium, gives regular compensatory motions on rotation about transverse axis, almost as great in degree as normal. Can detect nothing on other rotations. Eyes appear normal; are moved a good deal but in no constant way. *Next day.* Animal still very lively. *Never turns on back.* Makes what are perhaps horizontal circus motions when disturbed [*i. e.* swims round and round in the aquarium], but to either right or left; seems rather trying to escape than subject to forced motions. Motions seem perfectly co-ordinated. Animal stopped with head up against side of aquarium; eyes compensated properly, *i. e.* rotated anterior end ventrally, posterior end dorsally. Animal came to rest next minute in horizontal position; eyes also horizontal. Rotated slowly about either a transverse or dorsi-ventral axis accompanied by nearly perfect compensatory motions. About longitudinal axis, compensatory motion of fins perfect. Turned on back by hand, resists and quickly turns back.”

Case II.—“Medium sized dogfish. On right side—cut nerve of (1) anterior vertical canal, (2) posterior vertical, (3) horizontal canal; on left side—(1), (2), and (3) successively. Operation very free from blood. Everything visible. Immediately after operation, no reaction. Animal in shock, and eyes show no spontaneous motion when first put in aquarium. After half an hour is much revived. Eyes make spontaneous motions little different from normal. Perhaps irregular nystagmus. [But the normal dogfish often moves its eyes rapidly and irregularly.] On rotation in vertical planes about a longitudinal axis compensatory motions of eyes indistinguishable from normal. Pectoral fins show marked compensatory motion. Dorsal fin appears to move slightly. Body stiff; almost impossible to bend; is kept bent concave to left. Rotation from horizontal to head up about transverse axis, left eye rotates very markedly in opposite direction. Right eye slightly.”

In all these animals, both flounders and dogfish, it will be objected that the otolith was still intact and that the sense of sight remained unimpaired. Both objections are well founded so far as they express the anatomical condition of the animals. If it be true, as v. Cyon¹ quotes from Beer, that the dogfish is day-blind, the latter objection would lose much of its force, but Beer's statement is contradicted by

¹ Von Cyon: Archiv für Physiologie, 1897, p. 77.

Lee,¹ with reason as it seems to me, for dogfish swimming in a large pool appear to see well. It has never been claimed that the otolith could effect compensatory motions on rotation in horizontal planes. Such motions were clearly exhibited by the dogfish, mentioned as Case I, which had lost all its canals and ampullæ.

Stimulation of canals. — If the theory of the semicircular canals is an expression of fact, stimulation of any canal should lead to motion of the eyes in the plane of that canal. Since the time of Flourens, many physiologists have made this assertion until it has become recognized as a well-established physiological fact. Yet in the flounder and dogfish, at least, it is but partially true. Furthermore it would be expected, as Professor Loeb pointed out to me, that if there were any difference in the irritability of the canals, it should be in favor of the vertical canals. For rotation of a fish in vertical planes is followed by a larger motion of the eye than rotation in horizontal planes. This supposition also is not true. When I began work on the flounder I had no idea that the canals were different in sensitiveness or that motion of the eyes in the plane of one canal upon stimulation of that canal would be more difficult of demonstration than in the plane of another canal. When, however, I came to compare the results of the first few operations, it was noticeable that *stimulation of the (morphologically) horizontal canals never failed to call forth motion of the eyes in the plane of the canal, while stimulation of the vertical canals either gave no results or motions of an indefinite character.*

Even with a mind at that time favorable to the theory, stimulation of the vertical canals in these first experiments gave me nothing better than—to quote from my notes: "Slight motion of eyes, seemingly in plane of canal;" "About 30° from plane of canal;" etc. Most often the notes read: "Not clear," "Indefinite," "No results," "Rotation not in plane of canal," etc.

As soon as I had grasped the significance of the difference between the horizontal and vertical canals, I began a series of experiments to ascertain whether the differences so far observed were individual or universal. The canals were exposed in different sequence, sometimes beginning with the anterior vertical, sometimes with the posterior vertical, sometimes with the horizontal. Different means of stimulation—a blunt needle, a tuft of cotton, a weak electric current—were employed. The results were indubitable. The morphologically

¹ LEE, F. S.: This journal, 1898, i, p. 136.

horizontal canals of the flounder are much more sensitive, measured by the resulting compensatory motions, than the vertical canals. Stimulation of the vertical canals near the ampullæ often gave no results whatever; while the slightest touch upon the horizontal canal, even far from its ampulla, led to prompt and very marked motion of the eyes in the plane of the canal. Even through a layer of bone a slight pressure over the horizontal canal found instant response in motion of the eye in the plane of the canal. While working near the vertical canals one perceived far more eye-motion in the plane of the horizontal canals than in that of the canal which was being uncovered. Stimulation of the vertical canals seldom or never gave responses in the planes of the canals. On the other hand the stimulation of the horizontal canal always produced motion of the eyes in the plane of that canal.

A few experiments upon dogfish convinced me that the same is true there. The horizontal canals are much more irritable than the vertical canals. Stimulation of the horizontal canals leads to definite motion of the eyes in their plane. Stimulation of the vertical canals gives very indefinite and indifferent results.

Have not the authors who assert that each canal mediates motions in its own plane founded their claims chiefly upon observations on one canal alone, the horizontal? Have not the vertical canals given them many negative results? Probably if the horizontals were no more sensitive and gave no more definite results than the vertical canals, no one would ever have heard that stimulation of a semicircular canal causes motion of the eyes or head in the plane of that canal.

Extirpation of the otolith in the flounder. — The flounder should be the classical animal for this operation, because the otolith in this fish is a hemispherical body, very firm and massive, and about 1 cm. in diameter. It lies in a capsule entirely apart from the semicircular canals, and can be removed unbroken without the letting of a drop of blood. As is well known, the otolith plays an indispensable part in the equilibrium theory of the ear. Upon the changed stress relations of the stone should depend the retaining of compensatory positions when the orientation of an animal with respect to gravity is changed. An animal from which the otoliths had been entirely removed might be able, according to theory, to make a compensatory motion upon rotation in vertical planes provided the vertical canals remained intact, but it could not on being stopped in any ab-

normal position preserve the compensating organ in the position into which it had come during rotation. *Flounders from which both otoliths had been entirely removed showed persisting compensatory motions* upon rotation in vertical planes about either a dorsi-ventral or longitudinal axis. These motions were very plain, though sometimes a little more slow than normally. The compensating positions were retained so long as the fish was held in an abnormal position. To make sure that sight impressions had nothing to do with this, the optic nerves were cut in an individual from which the otoliths had already been removed. This operation is very easy, as the nerves are several centimetres long and easily accessible. After the severing of these nerves the compensatory motions were as clear and persisting as before.

Professor Loeb¹ several years ago instituted investigations on the otolith of the dogfish. He found that when he washed out the otoliths no disturbance followed. But if the otoliths were scratched out, marked disturbances of compensatory motions resulted. He concluded that in his first experiment parts of the loosely segregated calcareous material were left in the ear and were able to functionate. The experiments on the flounder, on the contrary, where the entire stone can indubitably be removed, make it probable that Professor Loeb's washing experiment was the better of the two, and that his results were against, rather than favorable to, the otolith hypothesis.

Extirpation of the otocyst in crustaceans.—The antennules of the crayfish were removed by means of small scissors. In each case a microscopical examination was made to determine whether the organ was entirely extirpated. In the latter part of the investigation it was found that the animal sustained the loss of the rostrum without inconvenience. By performing this operation a few days before removing the otocysts, the latter operation was made easier and surer.

The removal of one antennule led to a lessening of the compensatory motions of the eyes on rotation in vertical planes. There seemed to be, however, no constant difference in the two eyes in this regard. Few experiments were made.

The loss of both antennules was the subject of more extensive experiments. The compensatory motions on rotation in vertical planes were always lessened *but never entirely done away with*. The residue was measured by rotating the operated animals in Apparatus A,

¹ LOEB, J.: Archiv f. d. ges. Physiol., 1891, xlix, p. 175.

and varied greatly, being sometimes no more than 8 per cent and again as much as 50 per cent of the original amount. The amount of residual compensation increased for some days after the operation, but at length became stationary, and did not change again although some of the animals were kept nearly a year and moulted one or more times. No regeneration of the antennules took place.

The turning of the eyes in the animals deprived of otoliths was less regular than in normal individuals. Upon motion of the animal through a certain arc and return backward to the starting point the eyes did not return so exactly to their original position. Sometimes rotation in one direction would produce marked movement of the eyes, while the reverse would hardly produce any reaction. This fact I never have been able to explain, but it seems contradictory to the supposition that the residue of compensatory motion after removal of the otoliths is a visual phenomenon and perhaps points to a one-sided loss of muscular energy or to one-sided nerve injury.

The compensatory motion of the eyes and accompanying nystagmus on rotation about a dorso-ventral (vertical) axis were not lessened in animals from which the otoliths had been removed. The same was true of *Gelasimus* and other sea-crabs upon which the same operation had been performed.

Animals from which the otocysts had been removed showed a diminution of general activity. They were not aggressive, and when they did grasp anything with their chelæ, held on less tenaciously than normal individuals.

In this connection I may say that Kreidl's beautiful experiments in which *Palæmon* on moulting was made to place otoliths of fine iron in the cyst and forthwith became susceptible to an electro-magnet as well as to gravitation, have seemed to me the most important proof of the connection of the otolith with equilibrium. Since I began to doubt the theory, I have attempted twice to repeat his experiments, but so far without success. I was unable to catch *Palæmon* at the moment of moulting. I watched them nearly all night, but while those captured late in the evening were often half moulting, they refused to continue the process, or else were unable to do so in the smooth glass dishes in which I had placed them. Some I supplied with eel-grass, with no better result. With small crayfish I was more successful. I saw them take the powdered lodestone with which I supplied them and place it upon the bases of their antennules. In a preliminary trial they did not respond to a magnet. Unfortunately

a thoughtless student placed the animals with some large crayfish, and they were devoured before I had opportunity either to finish the experiment or make a microscopical examination of the cysts.

EXPERIMENTS ON THE EYES.

From the foregoing experiments and discussion it seems certain that the otolith and semicircular canals are not the only organs for the mediation of compensatory motions. The fact that such motions exist in insects is sufficient proof of this point. Either the motions have not in all classes of animals one common cause, or else the semicircular canal and otolith theory is false. It is worth while at least to search for a common organ. Sight is a common function of all animals in which compensatory motions have been observed, and the eyes are perhaps the commonest organs exhibiting these motions. Do all or any compensatory motions arise from stimuli reaching the brain along the optic nerves?

Von Cyon¹ has stated that a frog when its head is covered with a leather cap ceases to display compensatory motions upon rotation on a horizontal turntable. He thinks that many of the equilibrium phenomena which the Mach-Breuer adherents have localized in the ear are really aroused through impressions upon the retina. Others who have worked on compensatory motions have claimed their total cessation when in addition to destruction of the labyrinth the animal was blinded, thus admitting that a part at least of such motions was attributable to the organs of sight. Clark asserts the same in regard to *Gelasimus*, all compensatory motions disappearing when, in addition to loss of the otocysts, sight was eliminated by painting the eyes with lampblack and shellac. He says also that when the eyes of normal crabs were painted, the otocyst being intact, little or no diminution of compensation followed. Lee² found that the compensatory motions of the dogfish were not affected by extirpation of the eyeballs.

Cutting the optic nerves of dogfish and flounders.—The optic nerves of dogfish and flounders were severed. No marked change in the compensatory motions in any plane could be detected. There was perhaps slightly less tendency to retain compensatory positions on rotation in vertical planes.

In the dogfish this operation is accomplished without loss of

¹ VON CYON: *Archiv für Physiologie*, 1897, p. 65.

² LEE: *This journal*, 1898, i, p. 133.

blood or disturbance either of the brain or orbit. The animal is placed upon its back and held down with netting and cords. Artificial respiration is easily established by keeping water flowing through the gills; the mouth is held open; the optic nerves are easily accessible through the skin and cartilage of the roof of the mouth. In the flounder also, as has already been stated, the long optic nerves allow of cutting without injury to the brain or interference with the muscles of the eye or with other nerves.

Crustaceans. — *Loss of sight.* — The eyes were painted two or more coats with a mixture of shellac varnish, and lampblack. Crayfish thus treated always showed a diminution of compensatory motions on rotation in vertical planes, amounting to 10 per cent or more of the total. The loss was, however, never excessive.

The compensatory motion and nystagmus of the eyestalks of crayfish on rotation about a dorsi-ventral axis were not affected by loss of sight. In *Gelasimus*, on the contrary, the same reaction was entirely done away with after the eyes were painted. In crayfish from which the otocysts had been removed, and whose eyes in addition had been painted, very little compensatory motion remained on rotation in vertical planes. *Yet in every case a small amount could be detected*, — one to five degrees.

Effect of light upon compensatory motions. — From the foregoing paragraph it appears that in the light the compensatory motions of a crayfish must be greater than in the dark. Is this effect indirect, the larger eye motion in the light being merely an increased effort to retain a moving retinal impression? Or does the light in some way affect the motion itself? I made a good many experiments to try to answer these questions, and while they have perhaps little bearing on the broad subject of equilibrium phenomena, they may be allowable in an article which attempts to deal only with compensatory motions.

Effect of moving objects upon eye motions. — A cylinder of white cardboard was fitted over the board carrying the animal in Apparatus A so that it could be revolved about the same axis. This cylinder was painted with longitudinal stripes inside, and well illuminated by placing the open back toward a window. When the cylinder was revolved about the animal, the latter being at rest, the eyes followed the motion of the stripes, but never to any great extent. If, for example, the cylinder was revolved 90°, the motion of the eyestalks was only a small part of what it would have been had the animal itself been rotated through the same arc.

This experiment makes it clear that moving objects may influence the eye motions of crustaceans. Other experiments, however, convinced me that light itself can influence the amount of response, and that the different wave-lengths have not equal value in producing this effect.

Effect of light and darkness.—Apparatus A was covered by a wooden box blackened inside. The box was open in front to allow observation of the mirror scales, but projected well forward of the animal, and shut out nearly all light from the animal's eyes. A normal individual which upon rotation from 120° right to 120° left showed in the light a total eye movement of 63°, in the dark box moved the eyes only 51°. The usual difference found was 5° to 8°, always in favor of the light.

Differences could be detected in the amount of eye motion in dark and light portions of the room, and between that on bright and that on foggy days.

Effect of colors.—In another series of experiments Apparatus A was surrounded by a blackened box which projected well in front of the animal but was open for a space of 8 cm. around the animal's head. This space could be filled with glass of any color or covered with black cloth. Animals rotated in this box with the space open showed the same eye movements as in the open room; with space covered with opaque black cloth, the same as in the dark box just described; with space covered with blue glass, nearly and in some instances quite as great rotation as in the open room; with space covered with red glass, usually slightly greater rotation than in the dark but not so great as in the blue light. These effects were the same, no matter in what order the colors were used, provided a slight interval was allowed between the trials. Some of the results obtained were as follows:—

Animal No.	Animal rotated	Total rotation of eyes (average).			
		Daylight.	Blue.	Red.	Dark.
		degrees	degrees	degrees	degrees
43 . . .	From 120° R to 120° L.	56.5	55	52	47
45 . . .	" 90° R to 90° L.	37.5	35	28.5	29.5
48 . . .	" 120° R to 120° L.	52.5	52.5	51	46
49 . . .	" 120° R to 120° L.	53.5	51.5	45.5	43.5

The red glass used was for my eye much brighter than the blue.

Effect of light on position of eyestalks.—A normal animal was placed horizontally in the apparatus. The mirror scale and indicators were adjusted. The animal was so placed that the light from a window fell upon the left eye. Upon interposing an opaque object between this eye and the source of light, the eye moved 1° to 2½° towards the vertical. Upon removing the screen, it fell to its former position. Upon interposing a piece of red glass the eye behaved in the same manner as if an opaque object had been used, though the movement was in a few cases less. Using blue glass, there was usually no change in the position of the eyes, either on placing it before the eye or taking it away. Sometimes motions were detected, but they were not so great as those from red glass. In general the blue light acted like daylight, the red like an opaque body. That these effects were not the result of the eye's following the objects moving before it was shown in several ways. (a) If the opaque object be moved horizontally the vertical motion of the eye occurs, being, as already stated, upwards when the light is intercepted, and downwards when it is allowed to fall again upon the eye. (b) If a black card well illuminated on the side toward the animal be moved slowly up past the eye, the latter also moves up gradually as much as 5° or 6°. As soon as the card has passed so that the window light falls upon the eye, it drops back again 1° to 3°. If the black card be moved slowly downwards between the eye and the light, the eye may move down 1° to 2°, but instead of springing back when the card has passed by, the light adds its effect and it falls still farther. (c) If an iris diaphragm is used to shut off and admit the light, the effects are not different than with the simple cardboard screen.

In only two cases was the eye opposite the light seen to move. In these instances this eye moved downwards when the light was shut off, and upwards when the light was readmitted. This experiment did not succeed with all animals. About one specimen in four showed no change on interposing the black card.

This experiment seems to me of some importance, in connection with the theory of heliotropism. To account for the orientation of animals in a ray of light, Professor Loeb¹ has advanced the idea that the tension of the muscles is directly or indirectly affected by light. If, for example, an animal has one side directed to the source

¹ LOEB, J.: *Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen*, Würzburg, 1896.

of illumination, and we imagine that light weakens the tension of the muscles on this side or increases the activity of the unlighted side, it follows that, when the animal moves, it must turn towards the source of light and be positively heliotropic. *The experiment just described seems to show that light may cause such an unequal tension of associated muscles.* When light enters the eye of the crayfish, the eye-stalk is not held so high as in the dark. This must be due to inequality in tension of the eye muscles. Either the muscles which lift the eyestalks are weakened, or those which depress it are strengthened, or both are weakened or strengthened in an unequal degree. The effect, too, agreed point by point with the phenomena of heliotropism. The blue rays acted exactly like white light, while red was like darkness or very feeble light.

A further experiment to show that light could affect the amount of geotropic response was made as follows: a half cylinder of cardboard which could be attached to the board carrying the animal and revolved with it was constructed. This covering was painted black inside, and enclosed the animal all around save a lateral slit about four centimetres wide over the animal's eyes and two small holes in front through which the mirror scales could be read. These holes, even, although they were not visible to the animal, were closed during the turning. The only light which reached the eyes came through the semicircular slit in the cylinder, directly over them. The experiments were performed on the roof of the laboratory at a time when the cloudless sky presented a semicircular field of vision of quite uniform illumination and free from objects which might catch the eye. The other half of the field of vision was made as similar as possible by means of mirrors placed beneath the apparatus. The animal on being rotated with the slit open showed, as was to be expected, the same eye motion as if the cylinder were not present. The slit was then covered with a sheet of tissue paper through which considerable light could penetrate but no object could be distinguished. The motion of the eyes for the same rotation of the animal was less. Two and three thicknesses of the paper led successively to lessening of the amount of compensatory motion, the loss each time being 1° to 3° . Removal of the paper layer by layer led to corresponding increases in the compensatory motions.

As in this experiment the animal's field of vision revolved with it, and the only difference was the amount of light admitted by the successive layers of paper, it would seem that light can influence the

amount of compensatory motion independently of the field of vision. And so far as these experiments go, they would indicate that the greater the light, the greater the eye motion for a given rotation of the body.

Under the title *Heterogene Induktion* Noll¹ has collected many instances of the interrelation of stimuli in plants, and has discussed the phenomena at some length. He shows that not every form of irritability can be traced to one, single stimulating cause. For example, he gives the observation of Stahl² that the rhizomes of many plants, as *Adoxa moschatellina* and *Circea lutetiana*, alter their direction of growth upon being lighted. But strangely enough the phenomena are not of a heliotropic character. Light is not the immediate cause, for on a klinostat the reaction fails. In other words, light is able to influence geotropism. Similarly Schmidt³ found that on the klinostat leaves did not show the so-called heliotropic torsion. He thought that the torsion was due to one-sided over-weight, but Noll says that it is evidently due to a union of heliotropism and geotropism.

It may be that the peculiar relation of the compensatory eye motion in the crayfish to the light is a case among animals of Noll's heterogeneous induction. It surely looks as if the amount of light entering the eye, independent of any visual field, can influence the geotropic response of these animals. I am inclined to believe, however, that the effect of light is a more general one; that in the light the entire animal is tuned, as it were, to a higher key. The tension of the muscles of an animal in the dark seems lower. This shows itself in reflexes and voluntary actions other than the geotropic. Blinded crayfish seem to grasp objects with their chelæ less strongly than usually. Beekillers when blinded rarely exhibit the stabbing reflex. And insects generally live only a comparatively short time after blinding.

Effect of loss of sight on compensatory motions of insects.—The preceding statements lead naturally to the inquiry as to what effect blinding has upon the compensatory motions of insects. I made numerous experiments to settle this point. Houseflies cease to react on the turntable as soon as the eyes are blackened. Placed on

¹ NOLL, F.: Ueber heterogene Induktion, 1892, Leipzig, W. Engelmann.

² STAHL, E.: Berichte der deutschen botanischen Gesellschaft, 1884, ii, p. 383.

³ SCHMIDT, O.: Das Zustandekommen der fixen Lichtlage blattartiger Organe durch Torsion, Inaugural Dissertation, Berlin, 1883.

their backs they would remain there for a long time without making any effort to get up. But they would still crawl up a vertical surface, and if turned head downward showed a strong tendency to reverse their position. The same was true of the locust and potato beetle. This geotropic response has, therefore, nothing to do with the eyes, as was indeed to be inferred from Professor Loeb's¹ experiment in which cockroaches confined in a dark box were found upon the vertical walls and with their anterior ends directed upwards.

All other insects experimented upon were like the housefly, showing no reaction to the turntable after both eyes were painted.² The compensatory motions and nystagmus exhibited by the head of the beekiller also ceased with loss of sight. As a rule, also, insects when blinded show marked loss of equilibrium and often refused to fly or move in a normal manner. The eyes, therefore, in accordance with the supposition of Delage,³ seem to be the most important organs of equilibrium for these animals.

To sum up my experiments on insects, it may be said that while all the species tried showed compensatory motions of one or more forms, these motions ceased entirely when the eyes were thrown out of function. The most natural explanation would be that these motions were roused by the shifting of visual impressions. I am not entirely certain, however, that this is the true, or at any rate I am doubtful that it is the entire, explanation. Blinding affects these animals so profoundly that it would not seem at all impossible that something akin to heterogeneous induction finds place in their vital economy. The experiment of revolving the field of vision along with the insect I did not perform. It would be worth while. At any rate, on the theory of shifting visual impressions I am at a loss to explain the retained compensating position exhibited by the beekiller when turned head downward. If the rotation of a beekiller around a dorsi-

¹ LOEB: Sitzungsberichte der Würzburger physikalisch-medizinischen Gesellschaft, 1888.

² A certain beetle scratched the paint from its eyes by a lively motion of the front pair of legs, using right and left alternately. To prevent this happening again, these legs were cut off at the first joint. The animal did not give any sign of recognition of the loss, but continued to move the stumps as if scratching, although they could not come near the paint. Though the second pair of legs could easily have reached the eyes, no effort was made to use them for that purpose. This observation seems in harmony with recent work of Bethe on the supposed intellectual powers of insects.

³ DELAGE: Archives de zoologie expérimentale et générale, 1887, (2), v.

ventral axis is stopped the moment the head is turned to one side, the head is not kept in this position but is brought back immediately to the normal position. If when turned head downward the animal does not recognize the changed relation to gravity, why is not the head here also brought back to the normal position? Certainly it is not merely the weight of the head which causes the motion, for the reaction fails when the eyes are painted.

SUMMARY.

1. Every form of compensatory motion known to be exhibited by vertebrates is found also in invertebrates, and not only in those which possess otocysts, as crustaceans, but also in those without, as insects. If, therefore, these motions have one common organ, that organ is not the semicircular canals nor the otocysts, nor a union of these. It will be argued that the compensatory motions of insects are of a different character, being done away with by blinding, while those of fishes, for example, are practically independent of the sense of sight. I cannot do more than call attention to v. Cyon's observation that those of a vertebrate — the frog — are also dependent upon sight.

2. In the flounder, an animal in which a very great shifting of the eyes has taken place, and which has come to lie on one side so that the canals are turned 90° from their ordinary position, the regular compensatory motions of the eyes are present. The removal of a part of the canals, for example the vertical canals, has in several cases failed to eliminate compensatory motions in the plane of the extirpated canals. In one case removal of all canals left a large amount of compensatory motion. Removal of the horizontal canals in a dogfish did not eliminate compensatory motions in that plane. Removal of the otoliths from the flounder (even with the addition of blinding) did not eliminate the power of retaining compensating positions when the orientation of the animal with respect to the force of gravity was changed.

3. The (morphologically) horizontal or external canals in flounders and dogfish are far more sensitive than the vertical canals. Stimulation of a horizontal canal leads inevitably to motion of the eyes in the plane of the canal. Stimulation of a vertical canal seldom or never leads to definite motion of the eyes in the plane of the canal. Yet compensatory motions in the planes of the vertical canals are normally more active than in the plane of the horizontal. It is supposable that the connection of the horizontal canals with that part of

the motor mechanism which mediates motions in the plane of these canals is of no significance, and that while artificial stimulation of these canals causes these motions the latter normally arise from some other source.

4. Independent of the shifting of retinal impressions the compensatory motions of the eyes of crayfish are greater in the light than in the dark. The failure of such reactions in blinded insects is perhaps explainable on some such basis.

5. It is the rays of greater refrangibility which thus affect the motion of the eyestalks of the crayfish.

6. Even after both blinding and extirpation of the otocysts, crayfish continue to show a small turning of the eyes on passive rotation of their bodies in vertical planes.

I desire to thank Professor Loeb for suggestions in planning lines of work, and for ever-ready help in overcoming experimental difficulties.

THE FUNCTIONAL ADAPTABILITY OF AFFERENT NERVE FIBRES.

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ASSUMING that there is no essential difference in the nature of sensory and motor impulses, we might expect that the former if transmitted from the periphery to a muscle or group of muscles instead of to the central nervous system, would be capable of provoking muscular contractions. The problem thus raised may be solved experimentally in the following way. A sensory nerve root may be divided between its ganglion and the spinal cord or medulla and its fibres allowed to regenerate along the course of a divided motor nerve to a muscle. The stimulation of the peripheral end of the regenerated sensory nerve should now cause contraction of the muscles to which the nerve had found its way, provided that a physiological, motor connection had formed between nerve and muscle, and provided that the nature of the effect produced by a nerve—whether sensory or motor—depends on the kind of tissue in which its terminal fibres lie.

In the present research the left pneumogastric nerve was severed on the cranial side of the ganglion of the trunk, and the peripheral cut end was sutured to the peripheral cut end of the hypoglossal nerve. Two or three months later the left pneumogastric was divided just above the thorax. From fourteen to eighteen days after the second operation the vagus-hypoglossal nerve and the tongue were excised and the peripheral end of the pneumogastric portion stimulated.

The animals used were dogs, rabbits, rats, and one cat. Ether or chloroform anaesthesia was maintained throughout all operations, the dogs being given in addition full doses of morphia. Asepsis was observed as far as possible.

The details of the primary operation were as follows. In the dogs, the nerves were united by two or three silk sutures passed through the epineurium. In the other animals the nerves were sutured by means of the fine silver wire used for winding violin strings. The wire, sharpened at either end, was inserted into the centre of the cross section of each nerve, carried along its axis for a few millimetres (in the case of the pneumogastric through the ganglion) and pushed out through the epineurium: the ends of the nerves were then approximated, and each end of the wire clinched. The clinching was accomplished by passing the end of the wire through the eye of a needle, and rotating the needle 180° on its own axis, the other end of the wire being held with a pair of forceps. The wire was allowed to remain permanently.

Of the twenty-eight animals which survived the operation, nine rabbits and two rats died after periods of from one to seventy days. The remaining seventeen were examined, after intervals of from sixty-six to one hundred and twelve days, in the following manner: The animal having been anaesthetized, the tongue was examined for fibrillary and rhythmic contractions; the pneumogastric was then exposed in the neck just above the thorax, and in the majority of cases tied with a silk thread and divided below the ligature. At the instant of the ligation of the nerve a watch was kept for any movement of the tongue. The central end of the nerve was then stimulated, just above the ligature, by rapidly repeated induction shocks of varying strengths, the tongue being observed at the same time. If there was no movement, the tongue and the vagus-hypoglossal nerve trunk were excised for histological examination, and the animal killed. The results of these experiments are given in Table I.

Notes on Experiments in Table I.

Dog I. The pneumogastric nerve was neither ligated nor divided, but merely freed from other tissues for a short distance, and stimulated.

Rabbit II. The strong tetanus produced by stimulating the nerve below the ganglion may have been caused by a spread of current to the hypoglossa portion.

Rabbit V. Fibrillary contractions were originally absent but appeared after ligating the pneumogastric.

The rhythm consisted of a puckering or dimpling of the surface in small areas; and in some cases there occurred a contraction of the longitudinal muscles of the left side.

TABLE I.

Animal.	Days since first operation.	Fibrillar contractions.	Rhythmic contractions with	Contraction of tongue in response to stimulus of X-XIII nerve below ganglion.		Remarks.
				Mechan. stim.	Electr. stim.	
Dog I . .	112	marked .	expiration	none .	distinct.	
" II . .	98	slight . .	expiration	distinct	distinct.	
" III . .	93	{ left none . } { right slight }	inspiration	slight .	distinct.	
" IV . .	75	slight . .	none . .	none .	none .	killed.
" V . .	67	slight . .	?	?	distinct	
Cat . . .	83	slight . .	none . .	slight .	distinct.	
Rabbit I .	98	?	?	none .	none .	killed.
" II . .	97	none . .	none . .	?	strong	died.
" III . .	77	marked .	?	?	distinct	died.
" IV . .	77	{ rt. marked } { left slight }	?	none .	none .	killed.
" V . .	76	none . .	none . .	slight .	distinct.	
" VI . .	73	{ marked in } { small areas }	expiration	distinct	strong.	
Rat I . .	76	marked .	none . .	?	none .	killed.
" II . .	73	?	inspiration	none	none .	killed.
" III . .	77	none . .	none . .	slight .	distinct	
" IV . .	66	none . .	{ inspiration } { right side }	none .	none .	killed.
" V . .	67	?	?	none .	none .	killed.

If the stimulation of the central end of the pneumogastric nerve caused muscular contraction of the tongue, the external wound was closed, and the animal allowed to recover.

After an interval of from fourteen to eighteen days, to allow for the degeneration of any ascending fibres which might be contained in the pneumogastric, the surviving animals were examined as follows: During anaesthesia, the tongue was examined for fibrillary and

rhythmic contractions; the lingual nerve was then exposed, divided, and stimulated, and the effect upon the tongue observed. The pneumogastric was isolated as far as the ganglion, and the peripheral end stimulated; the nerve trunk, with its ganglion, was then dissected from the surrounding tissues as far as the tongue, and the peripheral end, ganglion, and hypoglossal portion stimulated in turn. The tongue with the X-XII nerve-trunk was then excised, and the nerve again stimulated. The results of this second set of experiments are given in Table II.

Notes on Experiments in Table II.

Dog II. Ninety-eight days after the first operation rhythmic movements of the tongue accompanied expiration; fourteen days after second operation they accompanied inspiration, and persisted after division of the lingual nerve and both pneumogastriacs.

Cat. On the eighty-third day there were no rhythmic movements of the tongue, but the rhythm appeared distinctly fourteen days after the second operation.

Rat III. On the seventy-seventh day there was no respiratory rhythm; it was present seventeen days after the second operation.

Rabbit VI. On the seventy-third day the rhythm accompanied expiration; sixteen days after the second operation it accompanied inspiration. In this animal the nature of the response on stimulating the X-XII nerve eighteen millimetres below the ganglion was such as to suggest that the current might have spread upwards to the hypoglossal portion of the nerve. An unusually strong response had, however, been obtained with a weak stimulus during the second operation when a spread of current was avoided. In the third operation there was a possible source of error in lifting the nerve in its entire length free of underlying tissues during stimulation: when thus raised and stimulated with a tetanizing current (of a strength barely perceptible to the human tongue) a marked contraction of the muscles of the left half of the tongue resulted. With a stimulus, plainly perceptible but not unpleasant to the human tongue, the left half of the rabbit's tongue lifted a weight of 70 grams. Stimulation of the lingual nerve with a current unbearable when applied to the human tongue resulted in a contraction capable of lifting barely 5 grams. With a painful stimulus applied to the X-XII nerve below the ganglion the contraction was sufficiently powerful to lift 300 grams just clear of the support. Two minutes after excision of the tongue and the nerve trunk, stimulation of the X-XII nerve below the ganglion produced precisely the same results. In other experiments, where a spread of current seemed possible, the results were controlled by crushing the nerve above the point stimulated and then repeating the stimulus as before.

TABLE II.

Animal.	Days since operation.		Fibrillar contraction.	Rhythmic contraction with	Lingual nerve stimulated.	X-XII nerve stim. above ganglion.	X-XII nerve stimulated below ganglion.				
	I.	II.					Stimulus.	Millimetres below.	Before dissection.	After dissection.	After excision.
Dog I .	126	14	marked	expiration	Yes . . .	Yes.	Electr.	50	Yes.	Yes.	Yes.
" II .	112	14	marked	inspiration	No . . .	Yes.	Electr.	50	Yes.	No.	No.
" III .	111	18	marked	inspiration	Yes . . .	Yes.	{ Electr. Mechan.	65	Yes.	No.	No.
							{ Electr. Mechan.	60	No.	Yes.	No.
Cat . . .	97	14	slight	inspiration	Yes . . .	Yes.	{ Electr. Mechan.	60	No.	Yes.	No.
							{ Electr. Mechan.	30	Yes.	No.	No.
Rabbit V	90	14	slight	none	Yes . . .	Yes.	{ Electr. Mechan.	20	No.	Yes.	No.
							{ Electr. Mechan.	10	No.	Yes.	No.
" VI	89	16	slight	inspiration	Yes . . .	Yes.	{ Electr. Mechan.	10	No.	No.	Yes.
Rat III .	94	17	slight	inspiration	No . . .	Yes.	Electr.	25	Yes.	Yes.	Yes.
							Electr.	18	Yes.	Yes.	Yes.
" .	94	17	slight	inspiration	No . . .	Yes.	{ Electr. Electr.	10	No.	No.	No.
							{ Electr. Mechan.	10	No.	No.	No.
Dog V .	86	19	very slight	inspiration	{ Yes, both electrical and mechanical }	.	{ Electr. Electr. Electr.	50	Yes.	No.	After death.
							{ Electr. Mechan.	160	Yes.	No.	5 min.
								200	No.	Yes.	5 min.
								60	No.	Yes.	10 min.

Dog V. In this animal, the pneumogastric having been left intact below the ganglion, unsuccessful attempts were made to provoke reflex movements of the tongue by electrical stimulation of the tracheal and laryngeal mucous membranes, and of the stomach, liver, and spleen. The failure was possibly due to the deep morphia narcosis, as no other reflex movements resulted though the right pneumogastric had suffered no injury. On opening the thorax, rhythmic movements of the tongue (previously well-marked) immediately ceased. Subsequent artificial respiration with the bellows caused no movement of the tongue. This point is discussed below.

During dissection of the thoracic portion of the pneumogastric, the heart stopped beating and failed to recover.

Five minutes later, after the X-XII trunk with its ganglion had been freed as far as the tongue, stimulation 16 cm. below the ganglion, with a current of moderate strength, caused marked depression of the left lateral border of the tongue. Ten minutes after cessation of the heart-beat, crushing the nerve 6 cm. below the ganglion produced the same result momentarily. It was then discovered that the mechanical stimulus within the thorax, which had proved ineffective, had been applied by mistake to the phrenic nerve and not to the pneumogastric as intended.

Heidenhain¹ observed that after degeneration of the hypoglossal, mechanical hyperæmia might cause the muscles of the tongue to contract. From some of the results obtained it seems probable that the rhythmic contractions which accompanied either inspiration or expiration in the majority of the animals used, were indirectly due to the changes of blood pressure accompanying respiration. The fact that in Dog I the rhythm continued after division of the lingual and both pneumogastric nerves makes it probable that it was not reflex. In Dog V the rhythm ceased when the thorax was opened and the mechanical effects of the blood pressure were no longer active.

In all cases where either the hypoglossal portion of the conjoined nerve trunk or the pneumogastric ganglion was stimulated, the response was more marked than that obtained on stimulating below the ganglion, and a weaker stimulus sufficed. Whether this was due to the regeneration of motor fibres may perhaps be determined by histological examination. The fact that fibrillary and rhythmic contractions continued may be taken as evidence that the regeneration of motor fibres was not extensive. If the nerve fibres responsible for the strong tetanic contractions, following stimulation of the hypoglossal, were outgrowths of the trunk ganglion cells, the persistence

¹ HEIDENHAIN: *Archiv für Physiologie*, 1883, Suppl., p. 170.

of fibrillation would indicate that they had not as yet acquired the full control exerted by normal motor fibres. However, in some animals fibrillation became more marked after the second operation; and in Dog V, whose pneumogastric was not divided, the fibrillations grew less and less. Furthermore, in Rabbit V, they appeared a few minutes after ligation of the nerve.

The contractions following stimulation of the pneumogastric below the ganglion were usually confined to the intrinsic muscles of the tongue, and were tetanic, but in most cases they were weak. Where the comparison was made, the contractions were, however, with one exception, more pronounced, more readily induced, and often more wide spread than those caused by stimulating the lingual nerve. They were not ordinary reflex movements, since they were not prevented by dividing all connection with the central nervous system, or by excision of the tongue. It may fairly be assumed that any ascending fibres contained in the pneumogastric had degenerated during the interval which had elapsed between the second and third operations. It would seem probable, therefore, that the reaction depended upon stimulation of the peripheral portion of such nerve fibres as were originally afferent to the central nervous system, but of which the portion central to the trunk ganglion cells had regenerated as far as the tongue, and had acquired a motor or pseudo-motor function.

Whatever may have been the connections made by their newly formed endings, these fibres appear to have assumed a function entirely different from that which they normally possess. There is, however, one reservation to be made, namely, that, although extremely improbable, it is still within the bounds of possibility that these fibres (which originally carried impulses from the periphery to the medulla) exerted an influence within the central nervous system similar to that exercised by the chorda tympani fibres within the tongue, by virtue of which the latter, after degeneration of the hypoglossal, assume a pseudo-motor function. Such hypothetical afferent fibres when deflected from the central nervous system, and carried to the tongue might establish connections precisely similar to those they had normally maintained, and by modifying the circulation, or the production of lymph, bring about contractions of the muscles of the tongue. The fact that no change in the color of the tongue during stimulation of the X-XII nerve was observed would tend to show that the contractions were not due to modifications of the circulation.

SUMMARY.

After division of the left pneumogastric nerve centrally to the trunk ganglion, the peripheral cut end was sutured to the peripheral cut end of the left hypoglossal. Two or three months later the left pneumogastric was divided just above the thorax. From fourteen to eighteen days after the second operation the X-XII nerve and the tongue were excised and the peripheral end of the pneumogastric portion stimulated. The muscles of the left half of the tongue responded with tetanic contractions, which were in most cases weak. We may conclude that afferent nerve fibres if deflected from the central nervous system and caused to reach a muscular organ may, when stimulated, cause contraction. It was not clear whether the nerve impulses transmitted by these fibres to the tongue were motor or pseudo-motor; but their function was evidently quite different from that which they normally possess.

The rhythmic movements of the tongue which often accompany either inspiration or expiration after degeneration of the hypoglossal nerve, are probably due indirectly to the rhythmic change of blood pressure.

COMPARISON OF THE EFFECT OF CERTAIN INORGANIC SOLUTIONS AND SOLUTIONS CONTAINING SERUM ALBUMIN ON THE RHYTHMIC CONTRACTILITY OF THE FROG'S HEART.

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THESE experiments were made to determine the relative effects of certain inorganic and organic solutions on the frog's heart, the ultimate object being to ascertain the nutritive influence, if any, of the albumins of blood, especially serum albumin. Several methods have been employed in the experiments and a brief description of these will be given before describing the results.

Method.—In the first few experiments the ventricle alone of the frog's heart was used. A Kronecker's perfusion cannula was inserted into the aorta and pushed down past the valves into the ventricle, where it was firmly fastened by a ligature placed in the auriculo-ventricular groove. The ventricle was then removed from the body of the frog and suspended in a tonometer which was filled with a 0.7 per cent solution of sodium chloride. The solutions, whose action on the heart was to be determined, were placed in bottles provided with Mariotte's tubes in order to keep the outflow under constant pressure. These bottles were mounted on an adjustable platform, and were connected with the perfusion cannula by rubber tubing. To facilitate the change from one solution to another a four-way stopcock was interposed; by means of this stopcock any one of four solutions could be fed to the heart. The ventricular beats were recorded by means of a water-manometer connected with the tonometer. In addition, the force of the heart-beat was registered from time to time by a second water or mercury manometer connected with the outflow tube from the heart. By clamping off this latter tube, the full force of the heart-beat could be recorded by the manometer.

This method did not prove entirely satisfactory, the ventricle alone being comparatively insensitive to some solutions which when used

upon the whole heart maintained a regular and long-sustained activity. In the later experiments, therefore, the method recommended by Howell and Cooke¹ was adopted, the whole heart being isolated in the following way. All vessels springing from the heart, except the left aorta and the inferior vena cava, were securely ligated. A small glass cannula was inserted into the left aorta and pushed down through the valves so that its end lay within the cavity of the ventricle. This cannula was fastened in place by a ligature round the left aortic arch, and served as an outflow tube from the heart. A short length of rubber tubing connected with the cannula enabled the observer to collect the liquid as it came from the heart. A similar cannula placed in the inferior vena cava served as an inflow tube. By means of rubber tubing, this cannula was connected with the four-way stopcock mentioned above. In this manner, a circulation was maintained through the entire heart, which was left in the body of the frog. The entire animal was placed in a convenient moist chamber so arranged that the circulation could be maintained for hours without injury to the heart from drying. In these experiments the ventricular beat was recorded, at first, by a light counterpoised lever, the foot of which rested upon the ventricle.

This method was not all that was desired, since the foot resting upon the heart was liable to change its position with variations in the tone of the muscle, and thus prevent a strict comparison of the amplitude of the beat when the heart was fed upon different solutions. Subsequently, Engelmann's method of recording the ventricular beat was used. A fine silk thread was fastened to the tip of the ventricle, and was then carried to a short upright piece projecting over the fulcrum of the recording lever. The lever was nearly counterpoised, and was so arranged as to magnify the contractions about ten times. Very satisfactory results were obtained with this means of registration.

The effect of a Ringer mixture after exhaustion upon saline and alkaline saline.—The original statement by Martius,² that the heart after complete so-called exhaustion by saline followed by alkaline saline, can be made to beat again only by liquids containing serum albumin, has been shown to be erroneous by Howell and Cooke, and subsequently by White.³ A heart after this treatment can in fact be made

¹ HOWELL and COOKE: *Journal of physiology*, 1893, xiv, p. 198.

² MARTIUS: *Archiv für Physiologie*, 1882, p. 543.

³ WHITE: *Journal of physiology*, 1896, xix, p. 344.

to beat for many hours if irrigated with a suitable Ringer mixture of calcium, potassium, and sodium salts. The correctness of this last statement was demonstrated in a number of experiments directed especially to this point. The following experiment may serve as an illustration.

Experiment Jan. 26, 1898.—The frog's heart isolated at 11.30 A.M. The heart irrigated with a solution of NaCl 0.65 per cent. The beats, at first feeble and irregular, improved greatly during the first ten minutes, and afterwards grew gradually smaller and ceased in about one hour. At this time, the heart was still sensitive to weak electrical stimulation (induction shocks). The solution was allowed to flow through the heart thirty minutes longer, and at the end of this time it was insensitive to electrical stimulation. A Martius alkaline saline (NaCl 0.7 per cent, Na_2CO_3 0.005 per cent) was then substituted for the simple saline. After six minutes the auricles began to contract and five minutes later there was a feeble contraction of the ventricle. The beats then improved in amplitude, and continued for one hour and fifty-five minutes. Ten minutes later, the heart had ceased to respond to electrical stimulation. A Ringer mixture (NaCl 0.6 per cent, Na_2CO_3 0.005 per cent, CaCl_2 0.01 per cent, KCl 0.0075 per cent) was then substituted. At the end of one minute, the heart began to beat and continued beating for seventeen hours and thirty minutes. The contractions at first were feeble but steadily improved during the first seven hours. At the end of this time the beats were almost seven times greater in amplitude than during the first hour and at least twice as great as in the most favorable period of the action of the Martius solution. Rhythmic tone waves were also exhibited during the entire period of seventeen hours.

In some of these experiments, the revival on the Ringer mixture was not so marked as in the case quoted, that is, was not maintained for so long a time. In others in which the alkaline saline was omitted, the Ringer mixture being used at once after complete standstill had been obtained on the neutral saline solution, the revival was, perhaps, more complete, indicating that the alkaline saline had no specific influence of a favorable character in developing the conditions for rhythmic pulsations. On the contrary, it would seem that to the extent it caused contractions of the heart muscle it contributed toward the development of the final condition of complete fatigue, which, as we shall see, occurs after prolonged action of a suitable Ringer mixture.

The relative effect of different combinations of inorganic salts in supporting the heart-beat.—An effort was made to determine by actual trial upon the isolated heart the most favorable combination of the

salts of calcium, potassium, and sodium. To this end, the following mixtures were tested either upon the same or different hearts.

1. NaCl 0.6 per cent — 100 c.c.	4. NaCl 0.7 per cent — 100 c.c.
CaCl ₂ 1 " — 1 "	CaCl ₂ 1 " — 2.8 "
KCl 1 " — 1 "	KCl 1 " — 3.5 "
NaHCO ₃ 1 " — 1 "	
2. H ₂ O — 1000 c.c.	5. NaCl 0.7 per cent — 100 c.c.
Ca ₃ P ₂ O ₈ — 260 mgrm.	CaCl ₂ 1 " — 2.8 "
Mg ₃ P ₂ O ₈ — 60 "	KCl 1 " — 3.5 "
KCl — 400 "	Dextrose 1 " — 1.0 "
NaCl — 7000 "	6. NaCl 0.7 per cent — 100 c.c.
	CaCl ₂ 1 " — 2.8 "
	KI 1 " — 3 "
	7. NaCl 0.7 per cent — 100 c.c.
3. NaCl 0.6 per cent — 100 c.c.	CaCl ₂ 1 " — 2.8 "
CaCl ₂ 1 " — 2.8 "	KCl 1 " — 3.5 "
KCl 1 " — 4 "	Na ₂ CO ₃ 1 " — 0.3 "

In this mixture not all the salts went into solution.

The following is a summary of one of the experiments in which several of these solutions were tested upon the same heart:—

Experiment. The heart isolated as usual was irrigated successively on neutral and alkaline saline solution, 0.7 per cent, until complete standstill ensued. After one hour and thirty minutes of this treatment the heart was quiet and insensitive to electrical stimulation. Solution 1 was then substituted with the result that the heart began to beat and continued beating for forty-five minutes. After irrigating for one hour, the heart refused to respond to electrical stimulation. Solution 3 was then substituted. After eight minutes, the ventricles were beating regularly and continued to beat for sixteen hours. Thirty minutes after the heart had ceased beating on this solution, it was found to be insensitive to electrical stimulation. Ten minutes later, solution 2 was substituted. During the first five minutes there was a gradual recovery of tone and after eight minutes the ventricle began to beat, and for thirty-five minutes continued to beat irregularly. Five minutes later the ventricle failed to respond to electrical stimulation. Sheep's serum diluted with two volumes of saline was next used. In two minutes the ventricle had begun to beat irregularly, subsequently it improved somewhat and continued to beat for three hours and ten minutes. Ten minutes later it failed to respond to electrical stimulation. At no time during this period were the beats so strong and regular as upon solution 3. Solution 4 was next used. The ventricle began to beat in two minutes, the beats rapidly improved in amplitude and rate and continued for a total of twenty-four hours. At the end of this period the heart was again insensitive to induction shocks. Sheep's serum diluted with two volumes of saline was again used. After forty minutes, the heart began to beat and con-

tinued for four hours. Solution 4 was then substituted for the serum. The ventricle after ten minutes again responded and continued beating for forty minutes. After this exhaustion, further attempts to revive the heart were unsuccessful.

In other similar experiments, solutions 5 and 6 were used. Both seemed to be injurious to the heart. This effect was distinctly marked after solution 6; irrigation for any time with this solution made it impossible to revive the heart subsequently with any of the mixtures given above, or with serum.

So far as these experiments went, the best effects were obtained with a Ringer mixture containing only NaCl , CaCl_2 , and KCl . This result is in accord with the conclusions reached by Howell¹ and Greene² in experiments performed in this laboratory. With regard to the best proportions for these salts, my experience agrees with that of the authors named, in showing that the solution employed by White did not contain the CaCl_2 and KCl in their most favorable combination. In some cases at least, an increase in the calcium in this, or indeed in other mixtures of the three salts, may be followed by a return of rhythmic pulsations in a heart that had come to a standstill. As an example of this result, the following experiment may be quoted.

Experiment. April 4, 5, 1898.—Frog's heart irrigated with neutral saline until standstill was produced. White's solution, solution 1, then substituted. The heart-beat upon this solution for eight hours. The irrigation was continued until the ventricle was insensitive to electrical stimulation. The proportion of CaCl_2 in the solution was then increased from 0.01 per cent to 0.02 per cent. The heart began to beat immediately, and continued to beat for nine hours and thirty minutes. Though still sensitive to electrical stimulation, no spontaneous beats occurred after this time. The proportion of CaCl_2 was again increased to 0.03 per cent. The heart again began to beat and continued beating for fifteen hours. After standstill had occurred upon this solution the proportion of CaCl_2 was raised to 0.04 per cent, causing a return of rhythmic contractions for twenty minutes. The amount of CaCl_2 was then increased successively to 0.05 per cent, 0.06 per cent, 0.07 per cent, and 0.08 per cent. At each addition there was a temporary revival only, and after 0.08 per cent the heart could not be revived by further addition of calcium chloride or by any of the solutions used in the experiments, or by blood serum or milk.

¹ HOWELL: This journal, 1898, ii, p. 47.

² GREENE: *Ibid.*, p. 82.

This experiment, like those reported by Howell and Greene, and the subsequent experiments recorded in this paper, show that White in testing the efficacy of solutions containing serum albumin erred in not first obtaining the maximum effect with inorganic solutions alone. Hence his deduction of a special nutritive action of serum albumin is inconclusive.

From my experiments as well as those reported by Howell and Greene it would seem that for the heart of the frog or terrapin the most favorable combination of the three salts is as follows, NaCl 0.7 per cent, KCl 0.3 to 0.4 per cent, and CaCl_2 0.26 to 0.28 per cent. In addition, a small quantity of Na_2CO_3 .003 per cent is required to give a favorable alkaline reaction, as will be shown in the experiments following.

The experiments previously described were mainly preparatory to the chief object of this investigation, namely, a comparison between the sustaining effect upon the frog's heart of a favorable inorganic solution and solutions containing serum or milk albumin. Having ascertained as nearly as possible the most favorable solution of inorganic salts, this comparison was then made with the following results.

The effect of blood-serum or milk following exhaustion upon a Ringer mixture.—In this series ten experiments were made with blood-serum and five with milk. In the first six of the serum experiments the heart was brought to a standstill by irrigation with 0.7 per cent sodium chloride solution. It was then supplied with a neutral Ringer mixture of the composition given above, but without the addition of the Na_2CO_3 . Irrigation with this solution was continued until the heart came to a standstill and was no longer sensitive to electrical stimulation. Sheep's serum diluted with twice its volume of normal saline was then substituted for the Ringer mixture. In every case there was a distinct and long-continued recovery of rhythmic contractions. In three of these cases after the serum had ceased to cause pulsations, the neutral Ringer mixture was again substituted; the result was a slight, short-lasting occurrence of feeble beats.

The revival of contractions in the ventricle by the use of serum after apparently complete exhaustion on the Ringer mixture cannot be referred, however, to the nutritive action of serum albumin, since the Ringer mixture in these cases showed a neutral reaction. In the last four experiments of the series, the hearts after being brought to a standstill on the 0.7 per cent NaCl solution was first irrigated

with a neutral Ringer mixture and after standstill had been obtained with this solution it was replaced by an alkaline Ringer mixture containing 3 c.c. of a 1 per cent solution of Na_2CO_3 to a litre. In every case the alkaline Ringer mixture revived the heart and caused it to beat vigorously. In these cases there was an increase also in the muscular tone of the ventricle. The recovery caused by the alkaline Ringer mixture lasted in every case for several hours. When the heart had finally come to a standstill on the alkaline mixture it was irrigated with sheep's serum diluted with two volumes of normal saline. The result obtained varied somewhat with the different hearts used. In two of the experiments, the serum did not revive the heart at all, in one it caused a short recovery lasting only twelve minutes, and in one there was a distinct recovery lasting for about two hours.

Similar results were obtained when diluted milk was used instead of blood-serum. After exhaustion on neutral and alkaline Ringer mixture the heart was fed with cow's milk diluted with nine volumes of a 0.7 per cent solution of NaCl . In only one of the five experiments did the milk cause a return of rhythmic contractions, and in this case only for forty-five minutes.

To further test the efficacy of the inorganic solutions as compared with serum and milk the procedure as given above was reversed. The heart was fed with blood or milk until standstill was produced and a suitable Ringer mixture was then substituted.

The effect of a Ringer mixture following exhaustion of the heart upon blood-serum or diluted milk.—Six experiments were made in this series. The hearts were first irrigated with normal saline 0.7 per cent until standstill was produced. They were then fed with sheep's serum diluted with two volumes of 0.7 per cent NaCl solution or with milk diluted with nine volumes of the same solution of NaCl , until the heart again refused to beat. From this condition the Ringer solution again revived the heart in every case, the revival lasting for from four to ten hours. The following is a summary of one of these experiments.

Experiment, March 14, 15, 1899.—Heart isolated as usual. At 9.25 P. M. March 14, irrigated with a solution of NaCl 0.7 per cent. At 10.20 P. M. the heart was quiet and insensitive to mechanical stimulation. At this time, the heart was irrigated with sheep's serum diluted with two volumes of NaCl 0.7 per cent solution. There was an almost immediate recovery of contractions, the

heart beating at the rate of 48 per minute. At 8.30 A. M. March 15 — after a period of nearly ten hours — the heart was beating slowly at the rate of seven per minute. At 10.15 A. M. the heart had ceased beating and at 10.25 was insensitive to mechanical stimulation. At 10.30 A. M. substituted a neutral Ringer mixture (NaCl 0.7 per cent, CaCl_2 0.28 per cent, KCl 0.35 per cent). The heart again began to beat. At 11.25 A. M. the rate of beat was 28 per minute. At 2.10 P. M. the rate had fallen to seven per minute. An alkaline Ringer mixture containing 3 c.c. of a 1 per cent solution of Na_2CO_3 to a litre, and with the CaCl_2 raised to 0.036 per cent, was then substituted. A marked improvement in the beat resulted, the rate increasing to 21 per minute. At 4.30 P. M. the rate was 14 per minute. The CaCl_2 was then increased to 0.05 per cent, causing a gradual improvement in the beat which lasted, however, but a short time. The heart then became gradually weaker and stopped beating at 8.20 P. M. At 8.25 P. M. the heart was insensitive to mechanical stimulation. The diluted sheep's serum was again fed to the heart, but no recovery of pulsations followed although the serum was allowed to flow through the heart for two hours.

In the above experiment it will be seen that after being washed with NaCl 0.7 per cent solution until it had stopped beating, the heart was made to beat on sheep's serum for a period of nearly twelve hours, and that it was then revived upon a Ringer mixture of inorganic salts and kept beating for about ten hours.

A similar result was obtained in three other experiments of the same series. In two other experiments, however, the heart after exhaustion on the Ringer mixture again showed a short recovery of spontaneous contractions when fed with diluted serum.

The effect of a solution of serum albumin upon the frog's heart. — In view of the oft-repeated assertion by Kronecker and his pupils that serum albumin has a special nutritive action towards heart muscle, it seemed desirable to attempt to isolate this proteid and test its effect upon the heart when dissolved in an isotonic solution of NaCl. Attempts were first made to prepare the serum albumin in crystalline form according to the method of Gürber. It was found impossible, however, by this method to obtain a solution of proteid entirely free from the ammonium sulphate used to effect the crystallization. Recourse was had, therefore, to a modification of a method described by Eichholz. The procedure was as follows. One litre of sheep's serum was saturated thoroughly with MgSO_4 at 30° C. to remove the paraglobulin. The serum was then filtered at 30° C. and the clear filtrate after cooling was decanted from the crystals of

MgSO₄ that deposited during the cooling. From this liquid the serum albumin was precipitated by the careful addition of a 10 per cent solution of acetic acid. The precipitate after settling was filtered off and brought into solution by the addition of one litre of water. The aqueous solution was then saturated with NaCl and the serum albumin again precipitated by the addition of 1 per cent HCl. This process was repeated and the precipitate finally obtained was dissolved in a litre of water and carefully neutralized with a 1 per cent solution of NaOH. In this way a solution of serum albumin was obtained containing an unknown amount of NaCl.

To bring this solution to the concentration in albumin of the diluted sheep's serum usually employed upon the heart it was diluted twice with saline and its osmotic pressure was then determined by the freezing point method. The result showed that $\Delta = 0.7498$ giving a molecular concentration therefore of 0.3988. The solution was consequently somewhat hypertonic as compared with the blood-serum of cold-blooded animals — since a determination of the concentration of the blood-serum of the terrapin in four cases gave an average result of 0.3929. The solution showed a heat coagulation of 77° C., gave no trace of calcium when treated with ammonium oxalate, and likewise no reaction for magnesium. A slight reaction, however, was obtained with Ba (OH)₂ showing the presence of a trace of sulphate.

This solution was tested upon the heart with and without the addition of calcium salts. The following summary of the experiment exhibits the results obtained.

Experiment May 8, 9, 1899. — The heart was isolated as usual, and at 5.15 p. m. May 8, was irrigated with a solution of NaCl 0.7 per cent. At 5.45 p. m. the heart had ceased beating and was insensitive to mechanical stimulation. At 5.46 p. m. the heart was irrigated with the solution of serum albumin described above. At 5.48 p. m. slight auricular contractions — the ventricle showed a marked increase in tone while the auricles were much dilated. At 7 p. m. a series of small ventricular beats lasting about five minutes. At 8.25 p. m. the heart was insensitive to mechanical stimulation. Began feeding alkaline Ringer solution. The heart commenced to beat almost immediately, the rate at 8.55 p. m. being 36 per minute. At 9.10 p. m. the heart was still beating well. The Ringer mixture was now replaced by the solution of serum albumin, to which, however, CaCl₂ had been added to the amount of 0.028 per cent and KCl to 0.035 per cent. The result was quite a marked increase in both the amplitude and the rate of the heart-beat, the heart

beating at 42 per minute. The tone of the ventricle was also improved. The contractions continued without interruption, at first strong and frequent but eventually decreasing in amplitude and rate for nearly eight hours. At 5 A. M. May 9, the heart had ceased to beat upon this solution but was still sensitive to mechanical stimulation. At 8.20 A. M. the heart was insensitive to stimulation. The alkaline Ringer solution was then fed with the result that the heart at once began beating. At 10.30 A. M. the rate was 30 per minute. At 11.30 A. M. while the heart was still beating well the original solution of serum albumin (without calcium chloride) was substituted. At 11.50 A. M. the heart had ceased beating. Changed again to the alkaline Ringer solution and the heart again began to beat. At 1 P. M. the heart was still beating. Substituted for the Ringer mixture, diluted milk (milk 1 vol. NaCl 0.7 per cent, 9 vols.) no change in the character of the beat could be detected. At 2.35 P. M. the heart had ceased beating and the alkaline Ringer mixture was again used. There was a slight revival of contractions upon this solution and at 3.45 P. M. the heart had ceased beating.

It appears to be clearly established by this experiment that serum albumin alone in isotonic (in this case hypertonic) solution of NaCl is entirely incapable of maintaining the contractility of the heart. A similar result has been shown by Howell and Cooke¹ and by Stiénon² to hold for paraglobulin in solution in normal saline.

In view of the results described in this paper and others that have been published from this laboratory (see papers by Howell and Greene) it seems futile to ascribe to serum albumin any special nutritive effect upon heart muscle. Blood-serum and diluted milk will maintain the contractions of an isolated frog's or terrapin's heart for long periods, but not longer than a suitable Ringer mixture, indeed not so long. And serum albumin alone is seemingly entirely neutral so far as arousing spontaneous contractions in the heart is concerned. Whether or not blood-serum contains any constituent capable of directly nourishing the heart muscle is not determined by these or the other experiments made in this laboratory, but two facts at least seem to be clearly established: First, that the heart contains, when isolated, a supply of contractile material capable of maintaining its contractions for long periods when the proper stimulating conditions are provided; second, that whatever nutritive action may be exerted by blood-serum upon the isolated heart is not referable to the serum albumin contained in it.

¹ HOWELL and COOKE: *Journal of physiology*, 1893, xiv, p. 198.

² STIÉNON: *Archiv für Physiologie*, 1878, p. 263.

SUMMARY.

The direct conclusions that may be drawn from the preceding experiments may be summarized briefly as follows:

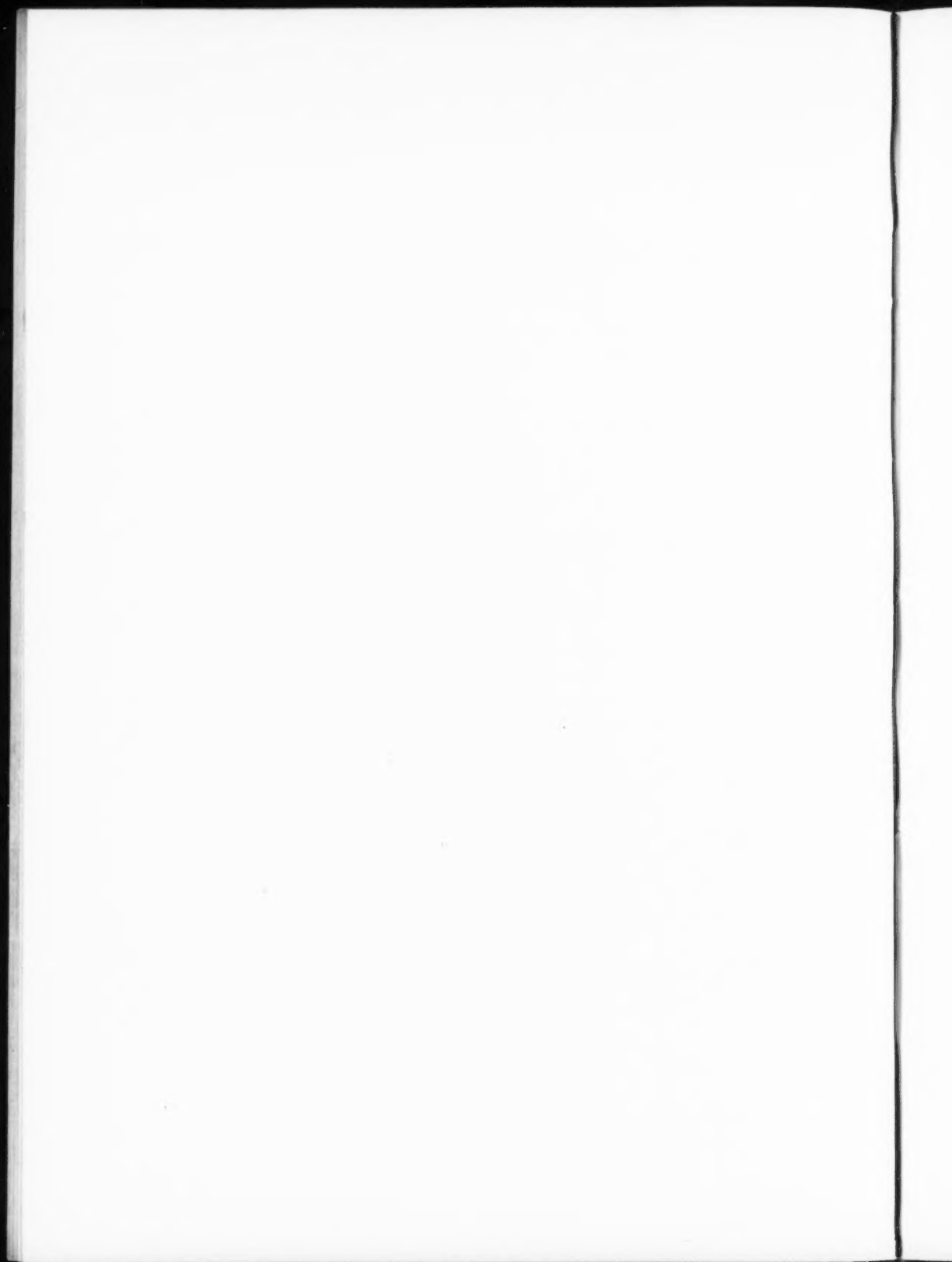
1. A frog's heart that has been brought to a standstill by successive irrigations of neutral and alkaline solutions of NaCl 0.7 per cent, may be made to beat again for many hours by the use of an alkaline Ringer mixture containing NaCl 0.7 per cent, KCl 0.035 per cent, CaCl_2 0.028 per cent, and Na_2CO_3 0.003 per cent.

2. When a frog's heart is brought to a standstill by the use of a neutral Ringer mixture a recovery of contractions may be obtained for a shorter or longer period by a gradual increase in the calcium contents of the circulating liquid.

3. A frog's heart thoroughly washed with neutral saline (NaCl 0.7 per cent) and subsequently brought to a complete standstill by prolonged irrigation with a suitable alkaline Ringer mixture does not usually show any recovery of contractions when fed with blood-serum or diluted milk. When recovery occurs it is feeble and short lasting.

4. A frog's heart thoroughly washed with neutral saline solution (NaCl 0.7 per cent) and subsequently brought to a standstill by prolonged irrigation with blood-serum (sheep's serum plus two volumes of 0.7 per cent NaCl solution) or diluted milk (cow's milk plus nine volumes of 0.7 per cent NaCl solution) usually shows a marked recovery of contractions when irrigated with a suitable alkaline Ringer mixture.

5. A frog's heart thoroughly washed with neutral saline solution (NaCl 0.7 per cent) cannot be revived by the use of serum albumin in isotonic solutions of NaCl. The addition, however, of suitable amounts of CaCl_2 to the solution of serum albumin will enable it to support the contractions of the heart as in the case of a Ringer mixture, blood-serum, or diluted milk.



ON THE NATURE OF THE PROCESS OF FERTILIZATION AND THE ARTIFICIAL PRODUCTION OF NORMAL LARVÆ (PLUTED) FROM THE UNFERTILIZED EGGS OF THE SEA URCHIN.

By JACQUES LOEB.

[From the Hull Physiological Laboratory of the University of Chicago.]

I. FORMER researches had led me to suspect that changes in the state of matter (liquefactions and solidifications) might play an important rôle in the mechanics of life phenomena. While studying the absorption of liquids by muscle I found that, to all appearances, a $\frac{1}{2}\%$ solution¹ of CaCl_2 favors the formation of solid compounds in the muscle, while an equimolecular solution of KCl favors the formation of more liquid compounds. Na -ions rank between the K - and Ca -ions. In these phenomena, however, much depends upon the concentration of the salts. We know that the enzymes of coagulation and liquefaction are greatly influenced in their action by the Ca -, Na -, K -, and Mg -ions. Ca favors coagulation and Mg does the reverse. Between these come the two other ions. In this case also much depends upon the concentration.

I have made a series of studies on the mechanics of life phenomena, which will be published shortly in this Journal. I wish now to deal only with one part of these studies, namely, that referring to the nature of the process of fertilization.

I found that in $\frac{1}{2}\%$ solutions² of CaCl_2 , NaCl , KCl , and MgCl_2 the segmentation of fertilized eggs of sea urchins (*Arbacia*) proceeded best in MgCl_2 , next best in KCl , while CaCl_2 proved to be the most injurious in the series.

Seven years ago I, and later, Norman, found that if the concentration of sea water be raised sufficiently by the addition of certain salts, a segmentation of the nucleus takes place without any segmentation of the protoplasm. Such eggs, however, when brought back into normal sea water, divide into as many cells as there are preformed

¹ I propose to substitute in the future the $\frac{1}{2}\%$ solution of NaCl for the 0.7 per cent solution. It is time that we were rid of percentage solutions in physiology.

² Approximately the concentration of sea water.

nuclei. This year I tried the effects of equimolecular solutions of $MgCl_2$, KI , $NaCl$, and $CaCl_2$ upon this process of nuclear division (in which the nuclear membrane is apparently liquefied), and found that the influence of the four salts (or rather kations) followed the order mentioned above.

We know that enzymes as a rule require a slight degree of acidity or alkalinity for their action. I showed last year that the addition of a small amount of H -ions to sea water retards or prevents segmentation, while a small amount of HO -ions favors and accelerates the development of the *Arbacia* egg.

2. It has been known for some time that the *unfertilized* eggs of echinoderms, worms, and arthropods begin to segment when left for a comparatively long time in sea water. This has generally been considered a pathological phenomenon. Mead succeeded in causing a segmentation of the unfertilized egg of a marine worm, *Chaetopterus*, by the addition of a very small amount of KCl to sea water. Morgan tried the effect of more concentrated sea water on the unfertilized eggs of sea urchins with results similar to those obtained by me previously with the same methods in fertilized eggs. If the unfertilized eggs are brought back from the more concentrated sea water into normal sea water, they break up into as many cells as there are nuclear masses preformed in the more concentrated solution. But in none of these cases did the cell divisions of the unfertilized eggs lead to the formation of a blastula. A heap of cells, at the best about sixty, were formed and then everything stopped. We cannot utilize these observations for the theory of fertilization, for the simple reason that the essential element of the process of fertilization, namely, the formation of an embryo, was lacking. In the case of tumors or galls we have cell division and even growth, and yet these cell divisions do not result in the formation of an embryo.

3. Some recent observations suggested to me that something in the constitution of the sea water prevented the unfertilized eggs of marine animals from developing parthenogenetically. Last year I found that the striped muscles of a frog beat rhythmically (like the heart) if put into a $\frac{1}{2}$ $NaCl$ or $NaBr$ solution. It is only the presence of K - and Ca -ions in the blood that prevents striated muscles from contracting rhythmically in the body. Romanes had observed that if the margin (with the nerve ring) in *Hydromedusæ* be cut off, the centre no longer contracts rhythmically. I found this summer that this is due solely to the presence of K - and Ca -ions in the sea water.

In a $\frac{5}{8}n$ solution of NaCl or still better of NaBr the centre continues to beat spontaneously. In applying this and my more recent observations on the relative influence of the various ions upon segmentation to the problem of artificial parthenogenesis it seemed to me that by making two changes in the constitution of sea water the eggs of the sea urchin might be able to produce perfect embryos without being fertilized. These changes were either a reduction of the Na- and Ca-ions or an increase in the Mg (or K) ions or both. I think that a great number of variations in this sense might bring about the desired effect, but the end of the season allowed me to try only a limited number of variations. Without going into details (which may be reserved for the full report) I will state briefly that the mixture of about 5000 $\frac{1}{8}n$ $MgCl_2$ with about 5000 c.c. of sea water was able to bring about the same effect as the entrance of a spermatozoon. The unfertilized eggs were left in such a solution for about two hours. When brought back into normal sea water they began to segment and form blastulae, gastrulae, and plutei, which were normal in every respect. The only difference was that fewer eggs developed, and that their development was slower than in the case of the normal development of fertilized eggs. With each experiment a series of control experiments was made to guard against the possible presence of spermatozoa in the sea water. Unfertilized eggs of the same female were brought into normal sea water, and in solutions with too little $MgCl_2$. Neither in the normal sea water nor in any of these solutions with too little $MgCl_2$ did one single egg develop into a blastula or show anything more than the beginning of a segmentation after a long time.

4. From these experiments it follows that the unfertilized egg of the sea urchin contains all the essential elements for the production of a perfect pluteus. The only reason that prevents the sea urchin from developing parthenogenetically under normal conditions is the constitution of the sea water. The latter either lacks the presence of a sufficient amount of the ions that are necessary for the mechanics of cell division (Mg, K, H₂O, or others), or it contains too large a quantity of ions that are unfavorable to this process (Ca, Na, or others), or both. All the spermatozoon *needs* to carry into the egg for the process of fertilization are ions to supplement the lack of the one or counteract the effects of the other class of ions in the sea water, or both. The spermatozoon *may*, however, carry in addition a number of enzymes or other material. The ions and not the nucleins in the spermatozoon are essential to the process of fertiliza-

tion (which may interest those who believe with me that physiologists ought to pay a little more attention to inorganic chemistry). I have no doubt that the same principles hold good for the process of fertilization of other, if not all, the marine animals, although the ions involved will probably differ in various species.

Finally we may ask the question, whether we may expect to produce artificial parthenogenesis in mammals. Janósik has found segmentation in the unfertilized eggs of mammals. This is similar to the fact mentioned above, that the unfertilized eggs of sea urchins may show a segmentation if they stay long enough in the sea water. I consider it possible that only the ions of the blood prevent the parthenogenetic origin of embryos in mammals, and I think it further not impossible that a transitory change in the ions of the blood may also allow complete parthenogenesis in mammals.

ON METABOLISM DURING A COMBINATION OF PHOSPHORUS POISONING AND PHLORHIZIN DIABETES.

By W. E. RAY, T. S. McDERMOTT, AND GRAHAM LUSK.

[From the Physiological Laboratories of the Yale Medical School, and of the New York University and Bellevue Hospital Medical College.]

RUDOLF VIRCHOW in 1858 divided fatty changes in the cell into two different classes: one a fatty degeneration in which cell substance became partially resolved into fat, the other a fatty infiltration in which fat from other parts of the body was transported to the cell.

The possibility of the origin of fat from proteid in fatty degeneration has at all times been defended by Voit and his school. Metabolism experiments by Pettenkofer and Voit¹ showed that on feeding large quantities of proteid, not all the carbonic acid is expired which belongs to the proteid destroyed, and this indicates a storage in the body of fat formed from proteid. This was further demonstrated in the later experiments of Cremer.² The value of these experiments has been denied by Pflüger,³ who claims that the calculations are incorrect.

Other proofs that fats may arise from proteids are the conversion of casein into fat in the ripening of cheese, and the transformation of muscle in a damp locality into a cheese-like mass called adipocere, which contains increased amounts of fatty acid. These two processes, however, take place under the influence of bacteria.

Quite recently Lindemann⁴ has shown that there may be a conversion of fat from proteid after death in a skin preserved in an antiseptic solution.

¹ PETTENKOFEER and VOIT: *Annalen der Chemie und Pharmacie*, 1862, li, Suppl. pp. 52-361.

² CREMER: *Münchener medicinische Wochenschrift*, 1897, xliv, p. 97.

³ PFLÜGER: *Archiv f. d. ges. Physiol.*, 1891, li, p. 229, and 1897, lxxviii, p. 176.

⁴ LINDEMANN: *Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie*, 1899, xxv, p. 392.

There remains finally the discussion of phosphorus poisoning.¹ There have been found, in many autopsies of people poisoned with phosphorus, fatty degenerations of the liver, of the kidney, voluntary muscle, and heart muscle.² Bauer³ confirmed this upon dogs, and noted a high rise in nitrogen excretion through the urine, indicating an increased proteid metabolism.

The poisons, arsenic, antimony, oleum pulegii, phallin, ietrogen, act in a similar manner. After Bauer's experiments showing the increased proteid metabolism in phosphorus poisoning, Falck⁴ tried to demonstrate that the influence of phosphorus was due only to the later stages of starvation, but Bauer⁵ in a second paper clearly proved that the rise of proteid metabolism in phosphorus poisoning takes place at a time when such a rise does not take place in starvation. Bauer found a rise in proteid metabolism in his first experiment as high as 295 per cent above the starvation normal. Such results have been obtained repeatedly by those investigating the subject, and recently Lo Monaco⁶ has obtained increases of 302 and 287 per cent in the proteid metabolism of fasting dogs after the administration of phosphorus. Only Athanasiu has failed to find any change in the proteid metabolism in phosphorus poisoning in his experiments upon frogs, and concerning this we will speak later.

The rise in proteid metabolism in phosphorus poisoning is only paralleled by that in phlorhizin diabetes. The rise represented by the figures 295, 302, and 287 per cent in phosphorus poisoning in dogs may be compared with 540, 450, 340, and 340 per cent found by Reilly, Nolan, and Lusk⁷ after giving phlorhizin to fasting dogs, although as may be noted the proteid metabolism in diabetes appears somewhat greater. Reilly, Nolan, and Lusk state, "indeed the cause of the high metabolism, namely, the non-burning of the carbohydrates, would seem to be identical in the two cases. In the case of diabetes the sugar is removed and its well-known sparing influence over pro-

¹ It is not our idea to enter fully into the literature of the question of the origin of fat from proteid, especially since this can be found in two recent articles: J. Athanasiu, *Archiv f. d. ges. Physiol.*, 1899, lxxiv, p. 511; and W. Lindemann, (*loc. cit.*).

² SCHULTZEN and RIES: *Annalen des Charité Krankenhaus*, 1869, xv, p. 1.

³ BAUER: *Zeitschrift für Biologie*, 1871, vii, p. 63.

⁴ FALCK: *Archiv f. exper. Pathol. u. Pharmacol.*, 1877, vii, p. 369.

⁵ BAUER: *Zeitschrift für Biologie*, 1878, xiv, p. 527.

⁶ LO MONACO: *Archivio di farmacologia e terapeutica*, 1896, iv, p. 373.

⁷ REILLY, NOLAN, and LUSK: *This journal*, 1898, i, p. 395.

teid metabolism is eliminated. The explanation of the similar metabolism in phosphorus poisoning which seems most plausible to us, is that the proteid sugar instead of being burned is converted quantitatively into fat with the resulting high proteid metabolism noted in fatty degeneration." Reilly, Nolan, and Lusk calculated that sugar could be obtained from the proteid in phlorhizin diabetes to the extent of 60 per cent of the whole proteid molecule.¹ Cremer,² indeed, taking into consideration the extractive nitrogen of the urine, calculates that the amount of sugar yielded may be as great as 68 per cent of the molecule. This same large sugar excretion has recently been discovered also by Rumpf³ in diabetes mellitus in man. Reilly, Nolan, and Lusk found in fasting and meat-fed dogs a constant excretion of dextrose in a definite ratio to nitrogen; the average ratio was D:N :: 3.75:1. Rumpf in one case of human diabetes on a meat diet gives figures from which it may be calculated that taking three different four-day periods the ratios ran 3.52:1; 3.87:1, and 3.66:1. In another case for eleven days the ratio averages 4.04:1, and later for ten days 3.85:1.

Our problem now is to show evidence that this sugar is converted in phosphorus poisoning wholly, or in part, into fat. It will be remembered that phlorhizin when first administered acts to sweep out the store of sugar from the body, and thereafter continued applications of the drug bring about a urinary excretion of dextrose and nitrogen in a fixed ratio approximating D:N :: 3.75:1. Hédon⁴ reports a case of phlorhizin diabetes in a dog, where with 11 per cent of dextrose in the urine, the quantity of dextrose in the blood was too small to be determined. This shows again that phlorhizin acts to sweep the body free of sugar. Our first idea was to produce phlorhizin diabetes in a dog, and subsequently to poison him with phosphorus. Under this latter influence it might occur that the sugar, instead of being eliminated through the urine, would be decreased in quantity there on account of its conversion into fat within the organism. The following is an experiment made in accord with these ideas:

¹ The priority of this discovery is attributed to v. Mering by Fr. Muller and J. Seeman (*Deutsche medicinische Wochenschrift*, 1899, p. 209). The reference concerning this is given wrongly and we believe v. Mering never put forward such a statement.

² CREMER: *Münchener medicinische Wochenschrift*, 1897, xliv, p. 97, and *Zeitschrift für Biologie*, 1899, xxxviii, p. 309.

³ RUMPF, T.: *Berliner klinische Wochenschrift*, 1898, p. 945.

⁴ HÉDON: *Comptes rendus de la société de biologie*, Paris, 1897, xlix, p. 60.

DOG I

1907	Amt. Urine c.c.	Weight, kilos.	Dextrose, grams.	Nitrogen, grams.	D : N	Extra D, grams.	Food.	Phlorizin every 8 hours in grams.	Remarks.
Nov. 14	94	10.4		2.80					Dog had fasted 36 hours.
" 15	362		33.78	4.09	8.28	18.44		0.5	
" 16	317		26.36	9.52	2.70			"	
" 17	408		36.72	9.63	3.81			"	
" 18	(445)		(29.66)	(7.03)	4.20			1	Some urine lost.
" 19	536	9.1	48.4	10.29	4.70	9.82	150 grams meat and 11.76 grams dextrose.	1	
" 20	571		41.52	10.89	3.81		150 grams meat.	1	
" 21	710		41.18	11.88	3.47		" " "	1	1 c.c. phosphorus oil.
" 22	700		43.26	12.30	3.32		" " "	1	4.15 p.m. = 1 c.c. phosphorus oil.
" 23	750		42.88	11.87	3.64		" " "	1	
" 24	(731)		(26.52)	(7.76)	3.43		" " "	1	Vomiting.
" 25	(158)	7.7	(2.15)	(0.70)	3.08		" " "	1	"

This dog fasted several days. Dextrose was fed with meat to demonstrate the quantitative elimination of the dextrose. The meat (150 grams per day) was continued, and on the seventh day of diabetes — the third day of meat feeding — 1 c.c. of a 1 per cent solution of phosphorus oil was injected subcutaneously at 8 A.M. On the following day at 4.15 P.M. the same amount of phosphorus oil was administered. The dog showed the symptoms of phosphorus poisoning: the urine soon became dark amber color from biliary pigment; during the last thirty-six hours there was frequent vomiting, and the vomit often became mixed with urine, which prevented its quantitative collection. The urine remained acid throughout the experiment.

Two points stand out sharply in the above experiment. The first is that the sugar excretion after giving phosphorus to the diabetic dog is practically unchanged. The second is that the nitrogen excretion is barely affected by phosphorus poisoning during phlorhizin diabetes. The figures might perhaps be interpreted to indicate a slight increase in the quantity of nitrogen, and a slight relative decrease in the amount of dextrose eliminated. It is however known that in phosphorus poisoning there is a largely increased destruction of cell nuclei, and this of itself might explain both the slight increase of nitrogen in the urine, and the slight decrease in the ratio D:N. This ratio, however, remains represented by the figures 3.47:1; 3.52, 3.61, and 3.43:1.

We have seen that the influence of phosphorus alone may cause a rise in proteid metabolism in the fasting dog of three hundred per cent. In the above case where phosphorus acts after the administration of phlorhizin the increase in the nitrogen in the urine is at most thirteen per cent. It has been pointed out that phosphorus induces a destruction of the cell nuclei; but that it *per se* attacks proteid destroying it, is not substantiated by any of our experiments. Throughout our experience we have found that the combination of phosphorus poisoning and phlorhizin diabetes will not give a materially higher proteid metabolism than phlorhizin diabetes alone. Therefore phosphorus does not act specifically to destroy proteid, but the proteid destruction is in consequence of metabolism in which a part of the proteid molecule is not burned. The fact that phosphorus does not prevent the elimination of dextrose in the usual quantity, does not invalidate the proposition that in phosphorus poisoning fat is produced from proteid metabolism, for under the influence of phlorhizin the sugar produced from proteid may be protected at once from burning before it has a chance of being converted into fat.

Perhaps the results would have been different if phosphorus were administered to an animal with pancreas diabetes (where the sugar collects in the body as it is produced).

One experiment of this sort has already been done by Baldi¹ with the following results:—

Dextrose.	Nitrogen.	D:N.	Remarks.
grams.	grams.		
13.82	8.58		300 grams meat daily.
18.81	10.46		
17.89	11.25		
27.74	16.36		
16.33	11.71		
15.32	13.11		
8.31	11.84		
13.63	11.11		
20.33	8.47	2.40	Phosphorus.
16.32	6.33	2.58	
28.29	9.56	2.96	
35.97	11.96	2.99	
16.78	10.44	1.60	50 grams meat eaten.
7.45	12.18	0.62	Food refused.
14.64	4.33	3.38	50 grams meat eaten.
13.36	5.90	2.26	Food refused.

It is unfortunate that Minkowski's ratio in pancreas diabetes of D:N::2.8:1 was not obtained in the first instance. Curiously enough the ratio was established as soon as phosphorus was administered and continued four days, until the symptoms of the poisoning, such as refusal of food, set in, when at once there was a diminution in the sugar excretion. The ratio here averages D:N::1.59:1, and it may be calculated that as much as 39 grams of dextrose has been retained in the body to subserve the purposes of the metabolism induced by phosphorus. Quinquaud² states that one month's administration of arsenic reduced the amount of sugar in the urine from 300 grams to 134 grams daily. Details of diet are, however, unfortunately not given. It is to be regretted that there are no further experiments in a direction in which investigation might be profitable.

It is possible that in phosphorus poisoning the sugar part of the proteid molecule may be quantitatively converted into fat. Under these circumstances the sugar of the blood and of the body fluids would naturally be destroyed, and the body would become sugar free. The

¹ BALDI: Archivio di farmacologia e terapeutica, 1894, ii, p. 490.

² QUINQUAUD: Comptes rendus de la société de biologie, Paris, 1882, p. 535.

disappearance of glycogen in phosphorus poisoning has frequently been remarked, although Rosenbaum¹ has found it present in muscle. If this condition of freedom from sugar really existed, then after administering phlorhizin, there would be no "extra sugar" to remove from the body, but the ratio D:N::3.75:1 would be obtained immediately. In a first experiment of ours this ratio was actually obtained. We have repeated the experiment many times and have failed in every instance to duplicate the first result. We have therefore modified our views, as will be shown hereafter. The success of these experiments would depend on the immediate action of phlorhizin after injection, and in fact we have demonstrated that a strong sugar reaction can be obtained in dogs from the washings of the bladder in from five and a half to six and a half minutes after the subcutaneous injection of phlorhizin.

The tables on pages 146-148 represent the work on six dogs.

A general survey of these tables shows a rise in proteid metabolism due to phosphorus amounting to 250, 260, 283, 248, 183, and 164 per cent. Addition of phlorhizin poisoning produced in four of the six cases a further rise in proteid metabolism from 250 to 484 per cent in Dog II, from 260 to 403 per cent in Dog III, from 183 to 215 per cent in Dog VI, and from 164 to 338 per cent in Dog VII. In Dogs IV and V where no such rise but a fall in nitrogen excretion took place, the dogs were in the later stages of phosphorus poisoning, and the kidneys were probably less active.

The general rise in proteid metabolism in phosphorus poisoning after giving phlorhizin would of itself tend to show that more of the non-nitrogenous portion of the proteid can be burned in phosphorus poisoning than can be burned in phlorhizin diabetes. So this alone would indicate that the sugar radicle is not quantitatively converted into fat. The results of giving phlorhizin to dogs poisoned with phosphorus are similar to those from phlorhizin alone. At first there is a sweeping out of "extra sugar" from the body, followed by the maintenance of the ratio approximating D: N:: 3. 75: 1. Only in the last stages of phosphorus poisoning, as in the cases of Dogs IV and V, are the figures variable, due perhaps to a failure of the kidney. Although during phosphorus poisoning the sugar is not quantitatively converted into other substances, *i. e.*, fat, tyrosin, leucin, etc., still we may believe that some of the sugar formed from proteid may be the source of such substances.

¹ ROSENBAUM: Archiv f. exper. Pathol. u. Pharmacol., 1882, xv, p. 450.

DOG II.

1917.	Amount of Urine, c.c.	Weight, kilos.	Dextrose, grams.	Nitrogen, grams.	D: N.	Drugs.	Remarks.
Dec. 2....	10.6	2.07	Dog had fasted 36 hours.
" 3....	65	2.40	1 c.c. Phosphorus oil.	
" 4....	101	3.17	Rise in N = 250 per cent.
" 5....	140	5.18	1 c.c. Phosphorus oil.	Rise in N = 484 per cent.
" 6....	400	36.48	10.02	3.64	1 gram Phlorizin every 6 hours.	
" 7....	250	21.04	6.28	3.37	1 " " "	
" 8....	8.6	Death.
" 9....	

1918.	Amount of Urine, c.c.	Weight, kilos.	Dextrose, grams.	Nitrogen, grams.	D: N.	Extra D, grams.	Drugs.	Remarks.
Feb. 27	6.9	Dog started fasting.
" 28	60	1.845	1 c.c. Phosphorus oil.	
Mar. 1	50	1.845	0.5 " " "	
" 2	80	2.312	
" 3	95	3.279	
" 4	115	16.60	4.801	1 gram Phlorizin every 8 hours	Rise in N = 260 per cent.
" 5	136	15.31	3.118	5.32	4.9	1 " " "	12 hour portions.
" 6	146	16.84	4.314	3.48	1 " " "	Rise in N = 403 per cent.
		16.84	4.702	3.58	

DOG IV.

1908.	Amount of Urine, c.c.	Weight, kilos.	Dextrose, grams.	Nitrogen, grams.	D : N	Drugs.	Remarks.
March 7	Started fasting.
" 9	48	8.2	1.69	1 c.c. Phosphorus oil.	8 hours urine 283 g. rise in N.
" 10	43	1.67	8 hours urine.
" 11	42	1.91	8 hours urine.
" 12	105	4.02	Dog weak, vomit. Temp. 37.
" 13	92	1.60	6.18	1 gram Phz. every 8 hours.	
	2	8.18	1.29	19.96	1 " " " 8 "	
	2	7.3	13.97	0.75			

DOG V.

1908.	Amount of Urine, c.c.	Weight, kilos.	Dextrose, grams.	Nitrogen, grams.	D : N	Drugs.	Remarks.
April 23	44	6.1	1.131	1 c.c. Phosphorus oil.	Dog very lively.
" 24	49	1.511	Rise in N = 248 g.
" 25	55	2.052	1 c.c. Phosphorus oil.	9 hours Rise in N = 154 g.
" 26	2	2.80	6.95	1 gram Phz. every 8 hours.	3 hours
	68.5	4.032	0.579	3.79	1 " " " 8 "	31 hours.
" 27	41	1.118	0.295	4.61	1 " " " 8 "	81 hours.
" 28	19	1.06	0.225	4.80	1 " " " 8 "	Calculated.
	19	5.1	0.555	0.115	
	

DOG VI.

1898.	Amount of Urine, c.c.	Weight, kilos.	Dextrose, grams.	Nitrogen, grams.	D:N.	Drugs.	Remarks.
May 8	226	22.3	3.33	Dog had fasted 2 days.
" 9	?	1 c.c. Phosphorus oil.	
" 10	380	3.44	3 hrs. 20 mins. Rise in N = 183 $\frac{c.c.}{g}$. 8 hrs. urine. Rise in N = 215 $\frac{c.c.}{g}$. 12 hrs. 40 min. urine. 6 hrs. urine. Dog died during the night. Last urine not analyzed.
" 11	600	4.52	1 c.c. Phosphorus oil.	
" 12	132 349 312	13.74 9.76	0.84 2.39 2.51	5.72 3.88	1 gram Phz. every 6 hours. 1 " " " 6 " 1 " " " 6 "	
" 13	57	21.3	1.05	0.30	3.54	1 " " " 6 "	

DOG VII.

1898.	Amount of Urine, c.c.	Weight, kilos.	Dextrose, grams.	Nitrogen, grams.	D:N.	Drugs.	Remarks.
June 3	136	17.23	3.70	Fasted 2 days. Urine mixed with faeces. Rise = 164 $\frac{c.c.}{g}$. 9 hours urine. 3 hours urine. 12 1/2 hours urine. Rise = 338 $\frac{c.c.}{g}$. 6 1/2 hours urine.
" 4	?	2.2 c.c. Phosphorus oil.	
" 5	5.57	
" 6	750	2.28	
" 7	370 90	16.18 6.95	1.68	7.05 4.14	1 gram Phz. every 8 hours	
" 8	?	30.00	8.79	3.40	1 " " " 8 "	
" 9	?	17.64	5.06	3.49	1 " " " 8 "	
" 10	

One observation has been made, namely, that after the administration of phlorhizin the urine quickly becomes acid. The urine during phosphorus poisoning is often found to be alkaline with ammonia, but this soon disappears after the administration of phlorhizin.

Münzer¹ has shown that during phosphorus poisoning certain acids are produced which tend to neutralize the blood, and the production of such acids tends to the formation of ammonia to neutralize them. If sodium carbonate be fed, the ammonia produced remains normal. In the rabbit, in which the organism has less power to produce ammonia to neutralize acids present, the administration of phosphorus does not induce the production of ammonia. Perhaps the acids are formed from the abnormal decomposition of the sugar part of the proteid molecule. The composition of the urine of animals poisoned with phosphorus does not greatly vary from the normal in containing a large proportion of its nitrogen as urea.²

The urine in phosphorus poisoning may also contain tyrosin and amido acids, notably leucin. The presence of these substances has been frequently reported in human urine,³ and they are always present in dog livers.⁴ Certainly large quantities of tyrosin were found in the urine of Dog VII. The dog had been poisoned with phosphorus and had then had phlorhizin diabetes for twenty-four hours, during which time tyrosin was excreted. This was followed by a diabetic urine containing no tyrosin that we detected. This may be interpreted to mean that the formation of tyrosin stopped with the abnormal decomposition of the non-nitrogenous remainder of the proteid molecule.

Frequently a clear yellow vomit was obtained from the dogs under the influence of phosphorus and phlorhizin, and we have one such from the case of a phlorhizin dog without phosphorus poisoning. These vomits are acid and usually turn green on standing, indicating the presence of biliary coloring matter. But we have never detected a trace of sugar. Levene⁵ has stated that the bile contains sugar in phlorhizin diabetes, but the quantity found was very small.

Post-mortem examination. — Rosenfeld⁶ has shown that dogs fed with sheep fat in abundance, deposit it in thick layers of reserve fat in the

¹ MÜNZER: *Centralblatt für klinische Medizin*, 1892, xiii, p. 489.

² MÜNZER, *ibid.*

³ FRÄNKEL: *Berliner klinische Wochenschrift*, 1878, p. 265.

⁴ BLENDERMANN: *Zeitschrift für physiol. Chemie*, 1882, vi, p. 234.

⁵ LEVENE: *Journal of experimental medicine*, 1897, ii, p. 107.

⁶ ROSENFELD: *Zeitschrift für klinische Medizin*, 1898, xxxvi, p. 232.

body, especially in the subcutaneous tissue, about the suprarenals, and the omentum. When one of these dogs was starved, the fat in the liver on the eighth day of fasting amounted to 12 per cent, which is about normal, and consisted of the normal dog fat. In another dog which fasted ten days, and during the last two was under the influence of phlorhizin, the fat in the liver amounted to 53.8 per cent and consisted largely of transported sheep's fat. The fat rises in the blood from 0.2-0.5 per cent (which is the normal in fasting) to 0.8-1.2 per cent under the influence of phlorhizin, and the blood plasma looks milky white. If carbohydrates be fed, or if phlorhizin be discontinued, the normal condition rapidly reappears.

Rosenfeld¹ has also shown that a similar transportation of sheep's fat to the liver may be brought about in phosphorus poisoning, and L. Daddi² points out that the fat in the blood is hereby much increased. Sandmeyer³ has noted a fatty change in the tissues of dogs made diabetic from the extirpation of the pancreas.

To these observations we can add the following of our own.⁴

In dogs treated with phlorhizin alone the most marked fatty changes were in the liver and kidney, but the heart muscle, thigh muscle, and diaphragm show fat globules arranged in parallel rows within the fibres.

In the dogs treated with phlorhizin and phosphorus together, the same phenomena occurred, except that within the liver, the columnar arrangement of the lobule was broken up, and there was decided fragmentation of the nuclei.

With phosphorus alone, the kidney shows some fat, but not so strikingly as with phlorhizin; the liver shows much fat with practically universal fragmentation of the nuclei. In two dogs that fasted fifteen days, and were under the influence of phosphorus for eleven of these days, the dried liver contained 37.93 and 41.80 per cent of fat, respectively. The fat was extracted according to the alcohol-ether-petroleum-ether method of E. Voit.⁵ The weight of the dogs at death was respectively 5.2 and 4.3 kilograms. The first dog died naturally, and the second was killed a few hours afterwards. In

¹ ROSENFELD: Congress für innere Medicin, 1897, xv, p. 427.

² DADDI: Archives italiennes de biologie, 1898, xxx, p. 437.

³ SANDMEYER: Zeitschrift für Biologie, 1892, xxix, p. 86.

⁴ We owe these to the kind assistance of Prof. C. J. Bartlett of the Yale Medical School, and Prof. E. K. Dunham of the Carnegie Laboratory, New York.

⁵ VOIT: Zeitschrift für Biologie, 1897, xxxv, p. 555.

both the symptoms of phosphorus poisoning were pronounced. Now although the kidney contained some fat, and the liver cells contained much fat with very thorough disintegration of their nuclei, the heart muscle appeared quite normal, there being in one case some fine granules which might possibly have been fat stained with osmic acid, and the voluntary muscle of the thigh appeared absolutely normal, without any fat whatever.

Throughout the experiments the ordinary oleum phosphoratum has been used except in the case of the last two dogs. Here the phosphorus oil was made up with about 10 per cent of ether, which prevents the loss of phosphorus by fuming. It likewise did not seem so poisonous. The small dogs each received on the fourth day of starvation 1 c.c., the same on the sixth day, 2 c.c. on the eighth, 1 c.c. on the tenth, and 2 c.c. on the eleventh, twelfth, and thirteenth days.

CONCLUSIONS.

It is now necessary to attempt to bring these facts to a plausible explanation. It is known that wherever a rise in proteid metabolism takes place there is a fatty change in certain organs. Phlorhizin diabetes prevents the burning of sugar in the body, and in consequence the proteid metabolism rises. If phosphorus be now given it will not produce any considerable further rise in proteid metabolism. By itself phosphorus does not act as direct destroyer of proteid, but it is reasonable to believe that the high proteid metabolism after giving phosphorus to dogs is alone due to perverted metabolism, to the non-burning of a portion of the proteid molecule which is stored within the body. Now in phosphorus poisoning the nitrogen from the proteid is largely excreted in the usual way,¹ with the exception of leucin and tyrosin, which may be stored in the liver or excreted through the urine. We have seen that the formation of leucin and tyrosin stops with the removal of the sugar radicle, through the administration of phlorhizin. They may therefore be considered as products of perverted metabolism. Whether fat itself is formed from proteid has not been proved by any of these experiments, although there is nothing inconsistent with that hypothesis.

We may perhaps explain the large transport of fat, by picturing a pathologically hungry cell,—a cell from which the sugar radicle has been entirely removed, as in diabetes, or in which the sugar radicle

¹ MÖNZER: *Centralblatt für klinische Medizin*, 1892, xlii, p. 489.

instead of being burned is being converted into fat, tyrosin, or other substances, as in phosphorus poisoning. Under these circumstances, a compensating food-stuff, in this case fat, is attracted to the cell in quantities greater than it can burn. If this be a true picture then the sugar-hungry cells of the liver, kidney, and muscle become filled with fat in phlorhizin poisoning, whereas in phosphorus poisoning the liver with its above described perverted metabolism attracts fat in the same way. If this changed metabolism takes place in the liver alone in phosphorus poisoning, and in the liver, muscles, and elsewhere in phlorhizin diabetes, it will explain why there is a greater rise in proteid metabolism in the latter case.

When Rosenfeld stopped the administration of phlorhizin to his fasting dogs, the normal cell metabolism returned and the fat disappeared from the liver. When he fed an excess of carbohydrates in phlorhizin diabetes, these carbohydrates could be burned as is indicated by the reduced proteid metabolism noted by others under similar circumstances,¹ and substantially the normal decomposition took place.

Athanasii,² it will be remembered, has shown that the total amount of fat does not increase in fasting frogs after phosphorus poisoning. But Athanasii finds no characteristic rise of nitrogen excretion, and no great rise of fat in the liver. Thus the livers of seventy normal fasting frogs contained 2.667 grams of fat, whereas seventy other frogs of equal weight and size, but poisoned with phosphorus, contained 3.494 grams of fat, a rise of thirty-seven per cent only. Leo³ finds that in frogs poisoned with phosphorus the fat may rise from the normal of 6.02 per cent to the phosphorus poisoned of 6.71 per cent. Leo at the same time found in the normal guinea pig 11.3 per cent of fat in the liver, in the poisoned one 30.8 per cent. In a rat the normal was 13.5 per cent of fat, and in the poisoned rat 32.2 per cent of fat were found. So in like manner three to four fold the normal quantity of fat has been found present in the dog's liver. It may be quite possible that the action of phosphorus in frogs is not the same as in mammals, so the experiments of Athanasii do not settle the question of the production of fat in phosphorus poisoning.

¹ MORITZ and PRAUSNITZ: *Zeitschrift für Biologie*, 1890, xxvii, p. 81.

² ATHANASII: *Archiv f. d. ges. Physiol.*, 1899, lxxiv, p. 511.

³ LEO: *Zeitschrift für physiol. Chemie*, 1885, ix, p. 490.

REMARKS ON THE THEORY OF THE PROTEID MOLECULE.

The idea that proteid contains a carbohydrate radicle to the extent of sixty per cent, has been criticized by F. Müller and John Seeman.¹ These authors in common with Kossel believe that the protamines are the basis of proteid bodies. Such a protamine is for example salmin $C_{60}H_{57}N_{17}O_6$, which through hydrolysis yields one molecule of histidin $C_6H_9N_3O_2$, one of lysin $C_6H_{11}N_2O_2$, and three of arginin $C_6H_{13}N_4O_2$. These are called by Kossel hexobases since they contain six carbon atoms. It is suggested that these hexobases, and especially leucin, which may be united with them, are the source of dextrose, which likewise has six atoms of carbon. Furthermore, Kutscher² has shown that prolonged trypsin digestion may break up proteid completely into leucin, tyrosin, lysin, arginin, histidin, ammonia, aspartic acid, glutamic acid, etc., and similar results have been obtained by Kossel after treating proteid 72 hours with 20 per cent hydrochloric acid. Such a cleavage carries with it in egg albumin, for example, eighty per cent of the available carbon, and does not indicate the presence of a carbohydrate radicle to the extent found by Reilly, Nolan, and Lusk. On the other hand since proteid is built up in the plant from sugar and amido acids, and since it is readily resolvable in the animal into sugar and substances burning to urea, and since no case is known where fatty radicles have been proved convertible into sugar in the body, we maintain that it seems more plausible that proteid is not in greater part built up of amido fatty acids, but that rather a carbohydrate or a carbohydrate-like radicle is united to nitrogenous radicles, perhaps such as the protamines. That sugars may be converted into fat by the animal is well established, and that fatty radicles might readily arise from the carbohydrate moiety in tryptic cleavage seems to us to be readily conceivable. The liberation of free ammonia in trypsin proteolysis indicates that profound changes may take place. Experiments by Cohn³ show that after feeding leucin to a rabbit the amount of liver glycogen may be slightly increased; but perhaps here the leucin in burning spares from combustion a small amount of proteid sugar.

The point of view determines the attitude in this case. It is no

¹ MÜLLER, F. and J. SEEMAN: Deutsche medicinische Wochenschrift, 1899, p. 209.

² KUTSCHER: Habilitationsschrift, 1899, Marburg.

³ COHN: Zeitschrift für physiol. Chemie, 1899, xlviii, p. 211.

more logical to say because in laboratory treatment proteid yields a large quantity of amido bases and acids containing six atoms of carbon, that therefore the carbohydrates obtained in metabolism must be obtained from these radicles, than it is to maintain because a large quantity of hexose sugar may be obtained in the metabolism that therefore a carbohydrate radicle may be considered a source of the hexo bases or acids.

SUMMARY.

1. In dogs diabetic with phlorhizin, phosphorus poisoning does not increase materially the proteid metabolism, nor change the sugar excretion.

2. In dogs poisoned with phosphorus the administration of phlorhizin causes the usual, preliminary sweeping out of the body sugars; then the establishment of the ratio between urinary dextrose and nitrogen approximating $D:N :: 3.75:1$; and it causes further an increase in the proteid metabolism. The urine changes from an ammoniacal to an acid reaction.

3. In one dog poisoned with phosphorus the administration of phlorhizin brought about an excretion of tyrosin crystals in the urine, which excretion shortly ceased.

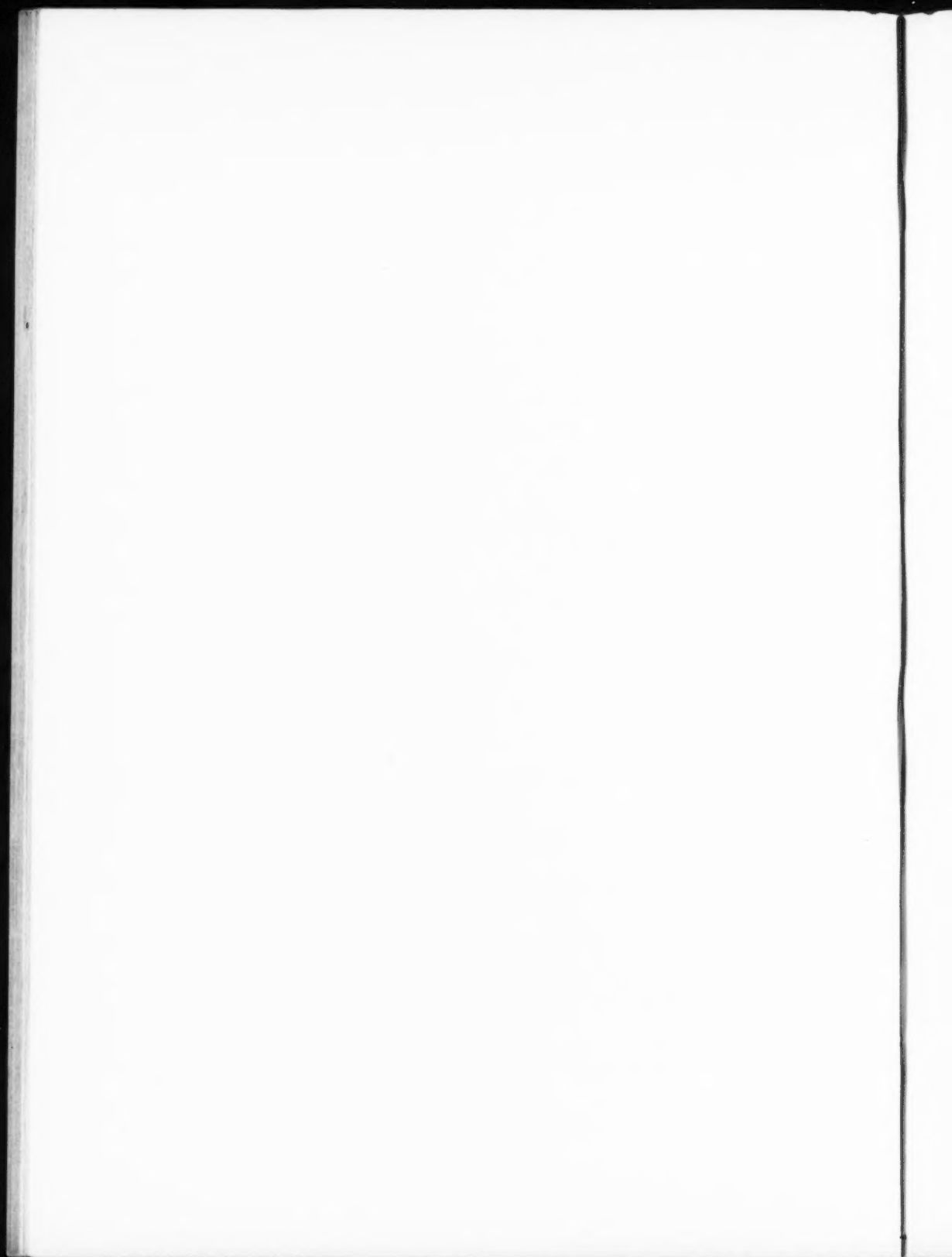
4. The vomit after phlorhizin diabetes was established never showed the presence of sugar.

5. The subcutaneous administration of phlorhizin dissolved in 1.2 per cent sodium carbonate solution, will bring about an excretion of sugar, which may be detected in the bladder washings in from five and a half to six and a half minutes after the injection.

6. The post-mortem examination of dogs poisoned with phlorhizin shows much fat in the liver, kidney, voluntary muscle, and heart muscle. In dogs poisoned with phlorhizin and phosphorus the same conditions are observed, and there is a decided fragmentation of the liver nuclei. In dogs poisoned with phosphorus alone, fat in large quantity was found in the liver, and there was a general fragmentation of the liver nuclei, while only a little fat was found in the kidney, none or almost none in the heart muscle fibres, and none in the voluntary muscle.

7. The following general explanation is offered. In phlorhizin diabetes the high proteid decomposition is due to the non-burning of the carbohydrate-like radicle of the proteid molecule. In phosphorus poisoning the high proteid decomposition is due to the conversion of

this carbohydrate radicle into leucin, tyrosin, and fat (fatty degeneration). Wherever the sugar from proteid is not burned are found pathologically hungry cells, which attract fat to themselves in larger quantities than can be utilized (fatty infiltration). Infiltration takes place generally in the tissues in phlorhizin diabetes; infiltration and degeneration take place in phosphorus poisoning, principally in the liver.



REACTION OF ENTOMOSTRACA TO STIMULATION BY LIGHT.¹

By R. M. YERKES.²

THE motor reactions of organisms to stimulation by light are of two kinds: first, reactions to the rays of light; second, reactions to the intensity of the light. The former is called phototaxis, the latter photopathy. Phototaxis is due to the directive influence of the rays, and may be either positive or negative. When an animal moves in the path of the rays toward the source of light, the reaction is said to be positively phototactic; when the movement is away from the source of light it is negatively phototactic. Photopathy is due to difference in light intensity. If an animal is in a vessel one portion of which is more intensely illuminated than another, it may move either from the less intense light to the more intense, in which case the reaction is positively photopathic, or from the more intense light to the less intense, this being a negatively photopathic reaction. When we speak of photopathic reactions the directive influence of the ray is supposed to be excluded, or so diminished that the intensity is paramount. It is obvious that in a phototactic movement the animal, while moving in the axes of the rays, is under the influence of change of intensity, but its movement is directed by the rays; whereas in photopathic reactions intensity determines the direction of movement.

PHOTOPATHIC REACTIONS OF CRUSTACEA.

Although crustaceans are very convenient forms for the study of reaction to light, comparatively little has been done with them. As early as the middle of the eighteenth century Trembley³ discovered that hydra seeks the light, as do also "pucerons," by which he probably meant some species of *Daphnia*. Trembley noticed that these animals collected on the side of a glass vessel next to a candle. When the light was moved the "pucerons" followed it.

¹ Contributions from the Zoological Laboratory of the Museum of Comparative Zoölogy at Harvard College, E. L. Mark, Director, No. 103.

² This is the first of a series of Psycho-physiological Studies planned by the author

³ TREMBLY, A.: *Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes*, Paris, 1744.

Loeb¹ states that the mud-inhabiting crab, *Cuma rathkii*, always moves toward the light side of a glass vessel. Thus even animals whose environment would lead us to expect no reaction to ordinary stimulation by light, react very definitely. This crab is markedly positively phototactic. It so orients itself, says Loeb, that the median plane of its body lies in the course of the rays.

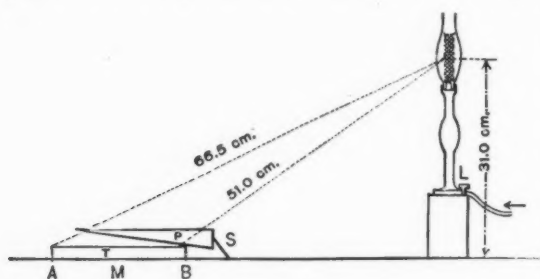


FIGURE 1. Diagram showing the position of apparatus and the direction of the rays in an experiment in phototaxis. *T*, trough of water containing organisms, *A* and *B* its two ends, *M* its middle. *P*, a prismatic box containing a solution of India ink. *S*, screen to cut off extraneous light. *L*, gas-lamp having a Welsbach burner. Drawn to scale. (After Davenport and Cannon.)

The reactions of some marine copepoda to light have been found by Loeb² to depend upon the temperature and concentration of the water. Herrick³ noted that the larva of *Homarus americanus* seeks the light. Evidently such observations are of little value so long as no measurements of the intensity of the light are made.

The larva is probably positively phototactic or photopathic at certain intensities and negatively so at others.

Two years ago Davenport and Cannon⁴ made experiments in this Laboratory which proved that the common fresh-water crustacean, *Daphnia*, is positively phototactic. The animals move from a region of greater intensity of illumination to one of less intensity in the direction of the light rays, *i.e.*, they move toward the source of light, even if this takes them into a darker region.

The animals were placed in a V-shaped trough (*T*, Fig. 1), and rays of light from the lamp, *L*, were passed through the prismatic box, *P*, to the trough. *P* contained a solution of India ink, and as the rays at *B* passed through a far thicker layer of the dark solution

¹ LOEB, J.: *Der Heliotropismus der Thiere, etc.*, Würzburg, 1890, p. 96.

² LOEB, J.: *Archiv f. d. ges. Physiol.*, 1893, liv, p. 91.

³ HERRICK, F. H.: *Bulletin U. S. Fish Commission*, 1895, xv, pp. 188-189.

⁴ DAVENPORT, C. B., and CANNON, W. B.: *Journal of physiology*, 1897, xxi, pp. 22-32.

than those at *A*, the portion of the trough marked *B* was less intensely illuminated. The rays, however, fell obliquely from *L* to *T* and it was observed that the *Daphnias* uniformly moved from the lighter region into the darker, in the direction of the entering rays. It was, furthermore, discovered that the rate of movement varied with the intensity of the light. But since the rate decreased only slightly with a great decrease in intensity, the experimenters concluded that the slower rate "is not the result of lower intensity, but that it is due to diminished precision of orientation showing itself in hesitating movements." The concluding statement of the account of the above observations, "That light acts principally through the direction of its rays," is one which further study may cause us to question.

In my own experiments two organisms were used. The first, *Simocephalus vetulus* Mueller, — also called *Daphnia vetula* by Baird and by Herrick, — is shown in Fig. 2. This according to Dr. Davenport was the animal used by him and Mr. Cannon. The second is *Cyclops parvus* Herrick. The animals were all obtained from little ponds near Fresh Pond, Cambridge. They are usually very abundant in slightly stagnant water and may be seen swimming near the surface.

Method. — To determine whether the organisms employed were photopathic they were placed in a trough from which all light was excluded excepting that which fell upon it from the side next to the source of light. The long axis of the trough was perpendicular to the direction in which the light came, so that the animal could not move in the direction of the rays. By passing the light through a prismatic box filled with India ink solution greater intensity was obtained at one end of the trough than at the other. Under these conditions any uniform movement toward the more or less intensely illuminated end would be a photopathic response.

The apparatus is shown in plan and section in Fig. 3. It consists of a V-shaped glass trough, *T*, 24 cm. long, 2 cm. wide, and 1.5 cm. deep, mounted upon a wooden base, *W*. Over the trough a frame, *F*, bearing five partitions, *p*, cut to fit the V-shaped trough, is so swung that it can easily be lowered in order to divide the space

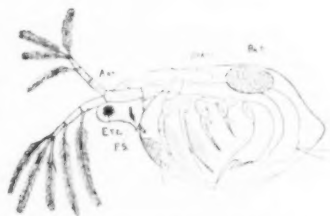


FIGURE 2. *Simocephalus vetulus*. *Ant.*, antenna; *D.G.*, dorsal gland; *Br.P.*, brood pouch; *P.S.*, pigment spot.

of the trough into six equal portions. *P* is a prismatic glass box 30 cm. long, 10.5 cm. wide at *B* and 8 cm. deep, filled with a solution of India ink. The three candles, *L*, stand 8 cm. apart, on a block of wood; the middle one being opposite the mid-

dle point of the trough.

Over the trough is a cardboard box, *C*, which is open on the side next the prism, *P*, to allow the light to fall upon the trough. *C'* is a large box covering the whole of the apparatus excepting the candles. The boxes serve to cut off light from the sides and top. At the end of *C'* next to the candles, *L*, is a screen of thin paraffin paper, *S*, to disperse the rays of light so that, if the India ink prism were removed, all portions of the trough would be uniformly illuminated. Since the light must pass through the prism, however, before reaching the trough, the illumination at the end marked + is greater than that at the end marked - (see Fig. 3 +

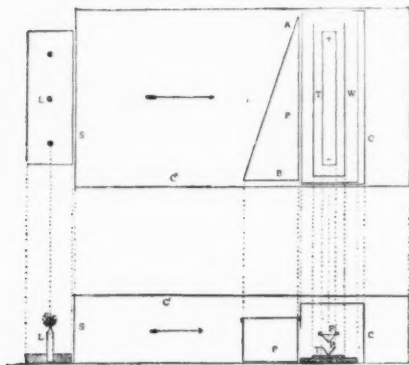


FIGURE 3. Plan and section of photopathic apparatus. *L*, candles; *S*, screen of paraffin paper; *C'*, large cardboard box; *P*, prismatic glass box containing India ink solution; *T*, glass trough for animals; *F*, frame bearing five partitions, one of which is shown in the section; *C*, cardboard box over trough; *H*, base of trough; *A* and *B* narrow and broad ends of *P* respectively; +, marks the end of *T* most intensely illuminated; -, marks the end least intensely illuminated. Arrows indicate direction of light rays. Drawn to scale.

and -). Thus the India ink solution secures a uniform decrease of light intensity in the trough from the + end to the - end.

The animals to be experimented upon were placed in the trough, an equal number in each of the six portions, or in as many as it seemed desirable to use, and the light from the candles, after passing through the screen, *S*, and the prism, *P*, fell upon them chiefly from the side. All other light was carefully excluded by having the apparatus in a dark room and by using the boxes *C* and *C'*. When everything was in readiness the partitions were raised by a simple mechanism and the animals permitted to move freely throughout the trough. After a certain time, usually three minutes, the partitions were lowered and the position of the animals determined.

Experiments with *Simocephalus vetulus* Mueller. — A certain number of animals were placed in the different compartments of the apparatus shown in Fig. 3. It was found that the animals placed in the end spaces sometimes got into the angles at the ends where the light could not affect them as it did those freely swimming in the trough. For this reason spaces numbered 1 and 6 in the tables, the end spaces, were abandoned. This tendency to seek the angles of the trough, or rather to remain in such places, is probably a thigmotactic reaction.

TABLE I.

Distance, 35 cm. Period, 3 minutes. Number of Animals, 4. Time, Dec. 20.

(+)	Lightest.			Darkest.			(-)
Spaces in trough. ¹	1	2	3	4	5	6	
Number of Animals. ²		1	1	1	1		Average.
Experiment 1 . . .	4	0	0	0	0	0	+2.5
" 2 . . .	2	2	0	0	0	0	+2
" 3 . . .	2	2	0	0	0	0	+2
" 4 . . .	3	1	0	0	0	0	+2.25
" 5 . . .	0	3	1	0	0	0	+1.25
" 6 . . .	2	1	1	0	0	0	+1.75
" 7 . . .	1	2	1	0	0	0	+1.5
" 8 . . .	2	1	0	1	0	0	+1.5
" 9 . . .	1	1	1	1	0	0	+1
" 10 . . .	1	2	0	1	0	0	+1.25
General average, +1.7.							
¹ The figures 1, 2, 3, 4, etc., in the first line of table refer to the six equal parts of the trough. In all the experiments space 1 was lightest; space 6 darkest. ² The second line of figures refers to the location of the animals at the beginning of each experiment. Throughout this series spaces 2, 3, 4, and 5 each contained one animal at the beginning of each trial.							

Table I. gives ten experiments under the conditions named at the top of table: Time 3 minutes, distance of light from trough 35 cm.,

number of animals, four. The trough, at the beginning of each experiment, was covered with the cardboard box *C*. After the animals had been allowed to swim freely in the trough for three minutes the frame was drawn down by means of a cord, the box, *C*, was removed and the number of individuals in each space counted. The results of these counts are given in each line of the table following the number of the experiment. In Experiment 1 of Table I., for example, the animals were all found in space 1. This indicates the maximum movement toward the intense light. The plus (+) sign at the top of table refers to the lightest end of the trough, movement toward this end is of course positively photopathic and is therefore marked +.

In order to express mathematically the result of an experiment, the mean position of the animals at the beginning of each experiment was compared with their mean position at the end of the experiment and the difference of the means was taken as an expression of the movement. In Table I. there was one animal in each of the spaces 2, 3, 4, and 5 at the beginning of the experiments. The mean position was therefore 3.5, but after Experiment 1, as the four animals were all in space 1, their mean position was 1. The amount of movement was then $3.5 - 1 = 2.5$, and since this was toward the lighter end it is +2.5. The four animals moved, as an average, two and one half spaces toward the more intensely illuminated portion of the trough. The mean, of course, is gotten by dividing, by the total number of animals, the sum of the products of each space into the number of animals.

Mean = $\frac{\text{Product of Space} \times \text{Number of Animals}}{\text{Total Number of Animals}}$. Applying this, in order to determine the mean in case of Experiment 2, we have $\frac{(1 \times 2) + (2 \times 2)}{4} = 1.5$; subtracting this from the original mean, 3.5, we obtain +2 as the amount of movement. When the mean position becomes greater than 3.5, there has been a movement toward the darker (−) end of the trough, and the result is therefore minus (−). All of the experiments of Table I. gave plus results. The sum of the positive movements in these ten trials is +17, which, divided by the number of experiments (10), gives us +1.7 as the average movement toward the positive end of the trough. This series therefore indicates a marked positively photopathic reaction.

The intensity of the light used in the foregoing experiments was

measured by Bunsen's photometric method. On opposite sides of a screen containing a transparent spot in the centre are thrown, at the same time, the light of a standard candle and the light from one of the candles of the apparatus which is made to pass through the screen of paraffin paper and the India ink solution before reaching the illuminated screen. The screen bearing a transparent spot is then placed at that point between the two sources of light at which the spot is least plainly seen. This is the point at which it is equally illuminated from both sides. The distance of each source of light from this point is then found and by means of the law of inverse squares (light intensity is inversely proportional to the square of the distance) the reduction in power caused by the paraffin screen and the India ink solution may be found. Fig. 4 shows the photometric apparatus.

Taking as our standard a paraffin candle burning

120 grains per hour, at a distance of 35 cm. from the surface illuminated, the value of the light for the first series would be obtained as follows. Two and one half cm. of the India ink solution (the quantity opposite space 1 of the trough) reduced the light three fourths. The paraffin paper reduced it one fifth. The light passing the paraffin-paper screen would then be four fifths of a candle power (*C. P.*) for each standard candle¹ used. The light passing the prism at space 1 would be one fourth of four fifths, which is one fifth *C. P.* Since three candles were used, the value of the light would be three times one fifth *C. P.*, or three fifths (0.6) *C. P.* The light falling upon space 6 of the trough passed through ten cm. of India ink solution; it would therefore be but one fourth of three fifths, or three twentieths (0.15) *C. P.* The amount of light at space 1 and space 6 respectively is, then, 0.6 *C. P.* and 0.15 *C. P.*

Since heat is an accompaniment of light it might be said that the so-called photopathic reaction was not due to light stimulation but wholly or in part to heat. To test this a series of observations



FIGURE 4. Diagram of photometric apparatus. *L*, lights; *P*, prism containing India ink solution; *S*, cardboard with opening for light; *s*, cardboard screen between prism and light; *M*, movable illuminated screen with transparent spot, *O*, in centre.

¹ The candles burned about 118.4 grains per hour.

(+) Spaces.		Lightest.						Darkest. (-)	
No. of Animals.		1	2	3	4	5	6	Average	
Experiment 1 . .	Period. 1 min.	3	1	1	2	1	0	+.875	
" 2 . .	1 "	3	1	2	2	0	0	+1.125	
" 3 . .	1.5 "	3	1	3	1	0	0	+1.25	
" 4 . .	2 "	3	4	0	1	0	0	+1.625	
" 5 . .	2 "	7	0	0	1	0	0	+2.125	
" 6 . .	2 "	7	0	0	0	1	0	+2.	
" 7 . .	2.5 "	5	1	1	1	0	0	+1.75	
" 8 . .	3 "	6	1	1	0	0	0	+2.125	
" 9 . .	4 "	2	2	1	2	1	0	+.75	
" 10 . .	5 "	2	1	3	1	1	0	+.75	
General average, +1.4375.									
CHECK EXPERIMENTS IN DARKNESS.									
Experiment 1 . .	3 min.	1	2	2	1	1	1	+.025	
" 2 . .	"	0	1	1	1	2	3	-1.125	
" 3 . .	"	1	1	3	1	2	0	+.025	
" 4 . .	"	2	0	2	0	3	1	-.625	
" 5 . .	"	0	3	1	2	1	1	0	
General average, -.025.									

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It is interesting to see the variations in the responses caused by changing the time of the experiment. The first two experiments were one minute in length, *i.e.*, the animals were allowed one minute to swim about the trough before the partitions were replaced. This time appeared to be too short for the full photopathic reaction. Two minutes gave a much greater positive response, as did also three,

TABLE III.

Period, 5 min. Distance, 35 cm. Number of Animals, 8. Time, Feb. 25.

(+)		Lightest.						Darkest. (-)	
Spaces.		1	2	3	4	5	6		
No. of Animals.			2	2	2	2			Average.
Experiment 1 . .		2	5	0	1	0	0		+1.5
" 2 . .		3	4	0	0	1	0		+1.5
" 3 . .		3	3	0	1	0	1		+1.125
" 4 . .		2	4	0	1	1	0		+1.125
" 5 . .		5	3	0	0	0	0		+2.125
" 6 . .		4	3	1	0	0	0		+1.875
" 7 . .		5	2	1	0	0	0		+2
" 8 . .		1	4	2	1	0	0		+1.125
" 9 . .		2	4	{ One } { killed }		0	1	0	+1.25
" 10 . .		2	3	2	1	0	0		+1.25
General average, 1.4875.									
One check experiment in dark gave - 0.125.									

When the time became longer than three minutes, the result showed a decrease. It seems probable that during the first two minutes all the animals have time to move to the most intensely lighted portion of the trough, *i.e.*, space 1. After moving about for a few seconds there they begin to move in the only direction possible, namely, toward the negative end of the trough. Whether this is due to a gradual lessening of the stimulation is uncertain. It might well be that after a few trips back and forth most of the animals would come

TABLE IV. — SERIES A.

Period, 3 min. Distance, 35 cm. Number of Animals, 8. Time, March 3.

(+) Lightest.		Darkest.					(-)
Spaces.	1	2	3	4	5	6	
No. of Animals.		2	2	2	2		Average.
Experiment 1	3	2	1	1	1	0	+1.125
" 2	4	3	0	1	0	0	+1.75
" 3	4	2	1	1	0	0	+1.625
" 4	6	2	0	0	0	0	+2.25
" 5	4	2	0	1	1	0	+1.375
" 6	4	2	0	1	0	1	+1.25
" 7	5	0	2	1	0	0	+1.625
" 8	4	2	2	0	0	0	+1.75
" 9	5	2	0	0	1	0	+1.75
" 10	6	1	0	0	1	0	+1.875
General average, + 1.6375.							
CHECK EXPERIMENTS IN DARK.							
Experiment 1	0	1	2	0	2	3	-1
" 2	1	3	2	0	1	1	+0.5
" 3	1	1	3	1	1	1	+0.125
" 4	2	1	1	1	1	2	0
" 5	2	0	3	0	2	1	+0.125
General average, - 0.05.							

to rest in that intensity of light most suitable for them, — that to which they are "attuned." It has been shown, then, that heat is not the controlling factor of the reactions, and that the most favorable time for getting a full reaction is from two to three minutes. A series of five check experiments, given at the bottom of Table II., very satisfactorily proves the absence of geotactic influences.

TABLE IV. — SERIES B.

Period, 3 min. Distance, 70 c.m. Number of Animals, 8. Time, March 3

(+)		Lightest.				Darkest.		(-)
Species.		1	2	3	4	5	6	
No. of Animals.		2	2	2	2			Average.
Experiment 1		2	2	2	0	2	0	+0.75
" 2		0	3	2	3	0	0	+0.75
" 3		5	0	0	1	2	0	+1.125
" 4		4	1	1	0	1	1	+1
" 5		1	2	0	3	2	0	+0.125
" 6		2	2	3	0	1	0	+1
" 7		3	1	3	1	0	0	+1.25
" 8		2	3	2	0	1	0	+1.125
" 9		1	3	2	0	2	0	+0.625
" 10		2	4	0	1	0	1	+1
General average, +0.875.								
CHECK EXPERIMENTS IN DARK.								
Experiment 1		1	1	2	2	1	1	0
" 2		1	1	1	1	2	2	0.5
" 3		1	2	1	3	1	0	+0.375
" 4		0	0	4	4	0	0	0
" 5		0	2	2	2	1	1	0.125
General average, 0.05.								

Table III consists of experiments like those of Table I. It shows a slightly less marked positive response.

It was my intention to try, at the conclusion of this series of experiments, a number of check or test experiments in total darkness, all

other conditions being the same. The first observation gave a result which seemed clearly to indicate that light was the determinant of the positive response. I was at this time unable to make any more tests, but from the results of many subsequent observations feel sure that gravity is not an important factor in these reactions.

TABLE IV. — SERIES C.

Period, 3 min. Distance, 105 cm. Number of animals, 8. Time, March 4.

(+) Lightest.		Darkest. (-)					
Spaces.	1	2	3	4	5	6	
No. of Animals.		2	2	2	2		Average.
Experiment 1	2	3	0	2	1	0	+0.875
" 2	0	2	2	3	0	1	0
" 3	2	1	1	2	2	0	+0.375
" 4	1	2	4	0	0	1	+0.625
" 5	1	2	1	2	2	0	+0.25
" 6	4	2	1	1	0	0	+1.625
" 7	3	2	1	1	1	0	+1.125
" 8	3	1	2	0	2	0	-0.875
" 9	4	1	1	0	2	0	+1.125
" 10	1	0	1	4	2	0	0
General average, +0.6875.							

What is the effect upon the positive photopathic response of *Simocephalus* of changes in light intensity? The experiments as seen in Table IV, series *A*, *B*, and *C*, partially answer this question. For series *A* and *B* the light intensity was the same as for Table I, *i. e.*: 0.60 *C. P.* at the + end of trough; 0.15 *C. P.* at the - end. The series is followed by five check experiments, the result of which proves conclusively that light was the controlling factor of the reactions.

The uniformity of the responses in all experiments thus far given is to be noted; out of thirty all have been positive. Table I. shows

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TABLE V.

Reactions in daylight. Cloudy afternoon. 4 P. M. Period, 3 min. Distance of trough from window, 70 cm. Number of animals, 8. Time, March 10.

(+)		Lightest.				Darkest.		(-)
Spaces.		1	2	3	4	5	6	Average.
No. of Animals.			2	2	2	2		
Experiment	1	1	3	1	2	1	0	+0.625
"	2	4	2	1	1	0	0	+1.375
"	3	5	0	0	2	1	0	+1.25
"	4	3	0	0	2	3	0	+0.25
"	5	2	1	2	2	1	0	+0.625
"	6 ¹	4	2	0	2	0	0	+1.50
"	7	1	2	0	3	1	1	0
"	8	1	1	3	1	1	1	+0.125
"	9	2	3	0	2	1	0	+0.875
"	10	4	1	0	0	2	1	+0.75
General average, 0.7375.								
¹ The last five experiments were made on other individuals some days later.								

a gradual decrease in the amount of positive response from + 2.5 to + 1.25; Table IV, *A*, however, gives just the opposite, a gradual increase from + 1.125 to + 1.875.

Series *B* contains ten experiments made under the same conditions as those of series *A* excepting a change in distance of the light. The candles were placed at 70 cm. from the trough, or double the former distance. The power of the light was therefore one fourth as great or 0.15 *C. P.* for the + end, 0.0375 *C. P.* for the - end.

The average of the series, +0.875 is a little more than half that of series *A*, which was + 1.6375. A decrease of the intensity to one fourth resulted in a decrease in the reactions by one half.

The final series, *C*, was carried out at a distance of 105 cm., which is three times the distance of series *A*. The intensities were then $0.066 + C. P.$ at + end; $0.016 + C. P.$ at - end. The result of the series, $+0.6875$, was a further decrease in the amount of positive

TABLE VI.

Responses to *Direct Sunlight*. Sunlight entering a west window was allowed to strike prism. Time, April. Hour, 3 P. M. Period, 3 min. Distance of trough from window, 70 cm. Number of animals, 8.

(+)		Lightest.					Darkest. (-)	
Spaces.		1	2	3	4	5	6	
No. of animals.			2	2	2	2		Average.
Experiment 1		4	1	2	0	0	1 <small>float.</small>	+1.25
"	2	1	2	1	3	1	0	+0.375
"	3	2	0	2	2	2	0	+0.25
"	4	0	2	3	2	1	0	+0.25
"	5	2	0	2	0	3	1	-0.125
"	6	2	1	1	2	1	1	+0.25
"	7	2	1	2	0	1	2	+0.125
"	8	1	1	0	3	0	3	-0.75
"	9	1	1	2	2	1	1	0
"	10	1	1	1	0	3	2	-0.75
General average, $+0.0875$.								

reaction. The reaction is not in this case, as it was before, inversely proportional to the distance. If it were, it would be $\frac{2}{3}$ of $+0.875$ or $+0.582$; but in fact the result is 0.1 greater than this.

From these data we are justified in concluding that, within certain limits, which it is my purpose to determine by further experimentation, the amount of the photopathic reaction of *Simocephalus* varies directly with the intensity of the light.

A few observations on the responses of *Simocephalus* to daylight and direct sunlight seem of interest.

The response to diffuse daylight, which was of course weakened by three fourths in being passed through the India ink solution, was definite and about the same as that obtained from the powers 0.15 *C. P.* and 0.375 *C. P.* of Table IV, series *B*.

Observation of animals in aquaria does not throw much light upon their preferences. I have frequently noticed that the majority would be on the side away from the window when the light was bright; but at other times they appear to be almost equally distributed.

The effect of direct sunlight is not surprising. Table VI. seems to prove that the light was almost too intense for a positive response.

Experiments with *Cyclops parvus* Herrick.—Having found that *Simocephalus vetulus* is, under ordinary conditions, positively photopathic I was interested to see whether another entomostrakon, from the same ponds, living, therefore, under the same external conditions, and in many respects similar in habits, would react in the same way. *Cyclops parvus* was the animal used. The experiments were made with the same apparatus and in the same manner as those with *Simocephalus*.

Table VII. gives first five check experiments made in total darkness to discover whether gravity or any factor other than light was influencing the animals. The result is so near zero that there is evidently no undesirable influence at work. Series I. includes ten trials, five of which were positive in result, four negative and one zero. The average of the series, + 0.075, is so slight a positive movement that we cannot feel certain of an inherent photopathic tendency of the organisms. Series II. is a continuation of Series I. with the light one fourth as strong, since the distance of the candles from the trough was doubled.

For the twenty experiments the same individuals were used. All had large ovisacs attached.

The average of the two series is zero, so we cannot say that *Cyclops parvus* responds to differences in light intensity as *Simocephalus* does. It seems probable, however, that to certain intensities of light *Cyclops* also would respond photopathically; if, for example, the light were exceedingly bright, it would not be surprising if the animals exhibited a negative tendency. It is also conceivable that at different seasons or in different stages of growth light would

General average, -0.075 . Average of Series I. and II. is 0.

TABLE VIII.

Period, 3 minutes. Distance, 70 cm. Number of Animals, 4. Time, April.

(-)		Lightest.					Darkest. (+)	
Spaces.		1	2	3	4	5	6	Average.
No. of animals.			1	1	1	1		
Experiment 1		0	1	1	1	0	1	-0.25
" 2		0	1	1	0	2	0	-0.25
" 3		1	1	0	0	2	0	+0.25
" 4		1	0	2	0	1	0	+0.50
" 5		0	3	0	0	0	1	+0.50
" 6		1	1	0	1	1	0	+0.50
" 7		1	0	1	1	1	0	+0.25
" 8		0	1	1	1	1	0	0
" 9		0	1	0	1	1	1	-0.75
" 10		1	1	1	0	1	0	+0.75
General average, + 0.15.								

affect the organism in different ways; for physiological conditions, as well as variations in the stimuli, determine the reactions of an animal.

Table VIII. consists of a series of observations made upon large brownish *Cyclops* without ovisacs. There is in this species a slight positive tendency, as six of the experiments give this result and the average is + 0.15, but this is not sufficient to justify the conclusion that the animals are photopathic.

I made almost a hundred experiments in addition to these with results varying from +1 to -1. One series of twenty observations gave - 0.04025, and another + 0.02025. It must be concluded in the light of this evidence that *Cyclops parvus* is not photopathic. So far as I know, no one has as yet determined whether it is phototactic.

PHOTOPATHIC COMPARED WITH CHROMOPATHIC REACTIONS.

Dr. Davenport in his *Experimental Morphology*¹ says "Thus both Bert² and Lubbock³ find that *Daphnia* accumulates especially in the yellow and green parts of the spectrum. Regarding these results I have only the comment that they need further confirmation." The confirmation of these results was the object of the following experiments.

TABLE IX.

Period, 3 minutes. Number of Animals, 12. Time, April 21.

Spaces.	1	2	3	4	5	6	
No. of Animals.		3	3	3	3		Average.
(-)		Blue.	Green.	Yellow.	Red-orange.	Red (outer-limit)	(+)
Experiment 1 . . .	0	0	1	7	3	1	+0.85
" 2 . . .	{ One } / killed }	1	1	3	3	3	+0.666
" 3 . . .	0	0	1	6	2	3	+1.083
" 4 . . .	2	0	0	8	1	1	+0.25
General average, +0.712.							

A large number of *Daphnias* were placed by Bert⁴ in a vessel from which all light had been excluded. An electric light spectrum was used to throw rays of any color desired into the vessel. When red, yellow, green, blue, or violet rays were used the animals gathered immediately in the portion of the vessel thus lighted. The responses to yellow and green were quickest. From this he concludes that *Daphnia* "sees all the luminous rays that we ourselves

¹ DAVENPORT: *Experimental morphology*, New York, 1897, i, p. 203.

² BERT: *Mémoires de la société des sciences physiques et naturelles*, Bordeaux, 1868, vi, p. 381.

³ LUBBOCK, J.: *Journal of the Linnean Society (Zoölogy)*, London, 1883, xvii, p. 214.

⁴ BERT: *Archives de physiologie*, 1869, p. 550.

see."¹ We may fairly ask here, May not their selection of the portion of the vessel lighted by the rays be a photopathic reaction to stimulation of the whole animal? This question will be discussed later.

To the heat end of the spectrum Bert got no response. Lubbock² also found that the animals always selected the red portion instead of the ultra-red or heat portion. "Certainly, therefore," Lubbock says, "their limits of vision at the red end of the spectrum seem approximately to coincide with ours." Thus he also assumes that the response is due to visual sensation. To ultra-violet Bert says there is no response. Therefore "Daphnia sees none of the luminous rays that we ourselves fail to see."³ This result Lubbock criticises. He found that the animals uniformly selected ultra-violet in preference to darkness. Hence, he concludes "that the limits of vision of Daphnia do not, at the violet end of the spectrum, coincide with ours, but that Daphnia, like the ant, is affected by the ultra-violet rays."

In Bert's experiments, when a complete spectrum was thrown upon the vessel, the Daphnias assembled in the yellow and green, a few only being in the orange, blue, and violet. These experiments are unsatisfactory because an inadequate description of the method is given and the general results alone are stated. In experimental work of this kind details of observations are of importance. Bert thinks the animals select the regions of greatest intensity. If so, this may be a purely photopathic reaction and not, as he assumes, chromopathic. Lubbock disagrees with Bert in that he thinks the animals do not "exactly follow the brilliancy of the light."

The following is a table from Lubbock.⁴

	Dark.	Violet.	Blue.	Green.	Yellow.	Red.
Experiment 1	0	0	3	39	5	3
" 2	0	1	2	37	7	3
" 3	0	0	4	31	10	5
" 4	0	1	5	30	8	6
" 5	0	1	4	33	6	6
	0	3	18	170	36	23

Although the yellow is the brightest part, there were more Daphnias in the green. In another series, however, the "yellow and lower

¹ "Mais voici déjà un premier point établi: les daphnies voient tous les rayons lumineux que nous voyons nous-mêmes."

² LUBBOCK: Journal of the Linnean Society (Zoölogy), London, 1881, xvi, p. 124.

³ "Les daphnies voient aucun des rayons lumineux que nous ne voyons pas nous-mêmes."

⁴ LUBBOCK: *loc. cit.*, p. 123.

green" contained most of the animals, so it is difficult to interpret Lubbock's statement that the *Daphnias* do not follow exactly the brilliancy of the light.

Engelmann¹ states on experimental evidence that *Navicula* prefers red and green to blue and violet. The percentages given are red 22.7, green 14.1, blue 6.9, violet 1.2. *Paramœcium bursaria* reacted to gas flame and sunlight spectra as follows:—

Gas.		Sunlight.
24.7 per cent	Red	9.7 per cent
23.3 "	Yellow	35.2 "
6.2 "	Green	14.6 "
5.3 "	Blue	25.5 "
0.8 "	Violet	0.8 "

Here there seems to be response to intensity.²

Lubbock does not describe the species of *Daphnia* used by him. Bert used *Daphnia puce*s, which I have been unable to obtain. My own experiments, about to be described, were made with *Simocephalus*. Our several results cannot therefore be compared without bearing in mind the possibility that even very similar forms may react differently to light. It should further be pointed out that Bert used the spectrum of the electric light, while my experiments, as will be seen, were made with a Welsbach gas burner; Lubbock does not state what light was employed by him.

Two problems present themselves for solution. First, does *Simocephalus* respond in any uniform and characteristic manner to a spectrum; second, is the preference for certain portions of the spectrum, when such exists, chromopathic (*i.e.*, response to the color of the light) or photopathic.

Method.—For the solution of the first problem the apparatus represented diagrammatically by Fig. 5 was used. Rays of light from a Welsbach gas burner, *L*, which was enclosed in a wooden box, *B*, passed through an adjustable slit, *S*, in the cardboard, *C*. By the lens, *l*, they were made parallel and thus fell upon the bisulphide of carbon prism, *P*. Since the red rays are least refrangible they fall upon the

¹ ENGELMANN, TH. W.: Archiv f. d. ges. Physiol., 1882, xxix, p. 390.

² It is well known, as Wundt (Human and animal psychology, translated by Creighton and Titchener, London, 1894, p. 348) states, that "ants try to escape from a violet light, but crowd together on a blue surface. Lizards and blind-worms avoid blue and all the more refrangible colours, but are fond of red." Some experiments which I have made to learn whether the pineal eye of lizards is functional prove that the animals will not select red light when their lateral eyes are covered.

mirror, *M*, at *R*, and the violet rays being most refrangible fall at *V*. The mirror reflected the spectrum upon the trough, *T*. It was necessary to have the rays perpendicular to the trough in order to avoid phototaxis, and this was most easily accomplished by a mirror. At first two bisulphide prisms of sixty degree angles were tried; with them it was impossible to get perpendicular rays. When the second prism was filled with water, the angle of refraction of which is less than that of carbon bisulphide, rays nearly perpendicular to the trough were obtained. The whole of the apparatus described was enclosed in a large box, *x*, one side of which was formed of black cloth so that the operator could easily reach the apparatus. During experiments all light was thus excluded

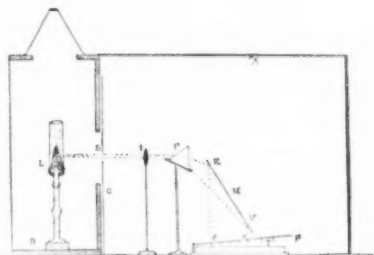


FIGURE 5. Diagram of apparatus used for experiments with a spectrum. *Z*, Welbach gas burner; *B*, box enclosing burner; *x*, large box around apparatus; *C*, cardboard; *S*, slit in *C* for transmission of light rays; *L*, lens and holder; *P*, bisulphide of carbon prism and holder; *M*, mirror to reflect spectrum upon trough; *T*, trough; *F*, frame bearing partitions; *R*, red end of spectrum; *V*, violet end of spectrum; *r-v*, extent of spectrum in trough. Dotted lines indicate course of rays. Drawn to scale.

from the trough, excepting that from the prism. The trough, *T*, was the same as that used for the photopathic work, but spaces 4 and 5 had been cut in halves by the insertion of two intermediate partitions, thus making four spaces each 2 cm. long. The spectrum used was about 10 cm. in length, so it extended at either end a little beyond the 2 cm. spaces.

Experiments. — In Table IX, are the data of four experiments, all of which indicate a decided preference for the yellow and red portions of the spectrum.

Ratio of number of animals in red-orange and yellow to number in blue and green for the four experiments is 8.25 : 1.

Ratio of number in red-orange to number in yellow is 1 : 2.66.

The spectrum for this series was obtained by the use of two prisms, one filled with bisulphide of carbon, the other with water. The rays were thrown upon the trough, not perpendicularly, but with an inclination of about 10° toward the red end of the spectrum. This removes the possibility of explaining the above reactions by phototaxis.

TABLE X.

Period, 3 minutes. Number of Animals, 12. Time, April 21.

Spaces.	1	2	3	4	5	6	
No. of Animals.		3	3	3	3		Average.
(+)	Red (outer limit).	Red- orange.	Yellow.	Green.	Blue.		(-)
Experiment 1	1	4	5	1	{ One / lost }	0	+1.167
" 2	1	4	5	1	1	0	+1
" 3	2	3	4	1	0	2	+0.5
" 4	0	1	6	3	0	2	-0.333
" 5	2	3	4	3	0	0	+0.833
" 6	0	4	4	2	2	0	+0.333
" 7	2	3	5	1	1	0	+0.833
" 8	1	4	4	1	2	0	+0.583
General average, +0.614.							

For the experiments of Table X. the apparatus of Fig. 5 was used, the mirror having been introduced in place of the water prism. All these experiments show a marked preference for yellow and red as before; but the yellow has a higher percentage of animals although not so high as in the first series.

Ratio of number in red-orange and yellow to number in blue and green is 3.3 +: 1.

Ratio of number in red-orange to number in yellow is 1: 1.4 +.

By these experiments Bert's conclusion that *Daphnia* reacts to the intensity of the different parts of the spectrum seems to be supported. More animals were found in the yellow and red-orange of the gas spectrum than in the other portions. I am satisfied that in the experiments tried by me the animals sought the most intense rays.¹

¹ I have taken Vierordt's (*Die Anwendung des Spectralapparates zur Messung und Vergleichung der Stärke des farbigen Lichtes*, Tübingen, 1871) measurements of intensity as the basis of this work. It is probable that his flames and mine would not give precisely the same results, but the differences would be too slight to impair the correctness of the above conclusion.

The second problem now presents itself. — Is this preference for orange and yellow which *Simocephalus* shows merely a reaction to intensity of light (photopathy) or to the color (chromopathy)? For this inquiry it was necessary to modify the apparatus somewhat. The spectrum was obtained in the manner shown in Fig. 5. The trough and its accompaniments used in these experiments were changed as shown in Fig. 6. The trough was covered with a cardboard box, *C*, having in the top a rectangular hole over which was placed a prismatic glass box containing India ink solution. The light passing through this solution at *B* traversed a greater depth of fluid than that passing at *A* and was therefore less intense.

To find whether the response was photopathic, it was necessary to decrease the intensity of the red end of the spectrum to such an extent that it would be equal to or weaker than the violet end. This was accomplished by the India ink solution. The greatest depth of the solution was placed over the red and yellow, causing a much greater decrease in the intensity of these than of the blue and green.

Experiments 1-5 of Table XI show a movement toward the violet. This was not very marked because there was almost equal intensity at different parts of the spectrum. It proves, however, that intensity is an important factor in the reaction. The plus or minus values of the experiments are given, but they have little significance because of the animals which passed beyond the limits of the spectrum. Immediately after this series of five I tried the animals (Exp. A) without the prism interposed, to see if they would react to the normal spectrum as before. The result left this indubitable; seven animals out of twelve were in the yellow.

Three thicknesses of paraffin paper were then placed over the whole trough and the spectrum allowed to pass through it (Exp. B). The result was a negative response, showing that the intensity of the spectrum had become too low for a definite reaction. One thickness of the paper was then used (Exp. C). In this case four sought the yellow

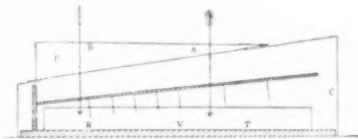


FIGURE 6. Diagram of apparatus for varying intensity of spectrum. *T*, trough; *C*, cardboard box fitting tightly about trough, with rectangular opening in top for admission of spectrum; *P*, prismatic glass box with India ink solution; *K-L*, indicates extent of spectrum. Arrows show direction of rays. *A*, end of spectrum at which intensity was least decreased; *B*, end of spectrum at which intensity was most decreased.

TABLE XI.

Period, 3 min. Number of Animals, 12. Time, April 25.

Spaces.	1	2	3	4	5	6	
No. of animals.		3	3	3	3		Average.
(+)		Red.	Yellow.	Green.	Blue.		(-)
Experiment 1	1	2	1	0	4	4	-0.833
" 2	3	2	1	2	1	3	+0.085
" 3	2	2	2	3	2	1	-0.167
" 4	2	2	1	3	1	3	-0.167
" 5	2	2	1	4	2	1	-0.083
General average, -0.166.							
Ratio of number in red and yellow to number in blue and green is 1 : 1.37 +. Ratio of number in yellow to number in blue is 1 : 2.							
Trial experiment without ink solution and screen interposed.							
Experiment A	0	1	7	4	0	0	
This shows a marked preference for the yellow, as do Tables IX. and X.							
Experiment with three thicknesses of paraffin paper over the whole trough.							
Experiment B	0	2	2	3	1	3	
Experiment with one thickness of paraffin paper.							
Experiment C	2	1	4	3	1	0	
Experiment with one thickness of paraffin paper over <i>Red</i> and <i>Yellow</i> .							
Experiment D . . .	0	2	1	6	3	0	
" D' . . .	0	1	1	8	2	0	

rays. The paraffin paper was next placed over the red and yellow only, leaving the green and blue at normal intensity (Exp. D). It was evident that the blue was thereby made brighter than the yellow. There was, moreover, a sharp line of demarcation between the yellow and blue where the screen ended. Under such conditions the response was very definite. All the animals but two were in the violet end of the spectrum, and eight were just beyond the edge of the screen, in the green. This, certainly, is strong evidence in favor of photopathy. It seems to indicate that the response of *Simocephalus* to the spectrum is photopathic and not chromopathic. I am not prepared to say that chromopathic reactions never occur, but it seems clear that for *Simocephalus* photopathy plays the chief rôle.

There is a further question which should have been fully answered by this research, but to which I have been unable to give any special attention. I refer to the problem: Is the response of *Simocephalus* to light due to stimulation of the eye (see Fig. 2) or to its direct effect upon the general body surface? In other words, is the process specific or general? As is well known, eyeless animals sometimes respond to light as definitely as those having organs of vision. Loeb¹ states that the eyeless larva of *Musca vomitoria* responds to light. Bert, as has been remarked, assumed that the movements of *Daphnia* were due to optic stimulation. This point I hope soon to examine; at present, I have a single observation which seems to bear upon the question. When *Simocephalus* was placed on the stage of a microscope and light was directed upon it from below, all side illumination being cut off by a hood of black cloth which was tied around the objective, the animal either swam about slowly or remained at rest. When at rest its appendages were always kept in rhythmic motion. A shadow thrown across the side of the field opposite to the animal when it was in this condition caused a momentary cessation of the rhythmic motion. This could be repeated three times, usually, before the animal became accustomed to the shadow and no longer ceased its activity. Evidently the general surface could not have been greatly affected by the shadow; it must have been sensed by a special organ of vision. This apparently supports Bert's assumption. It does not, however, prove that the eye is the only part stimulated, for although such a special organ for light perception as is possessed by *Simocephalus* or *Daphnia* probably is in this case the chief source of sen-

¹ LOEB: *Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen*, Würzburg, 1890, p. 111.

sation, the general body surface may also be affected to some extent and contribute to the reaction. It seems likely that, as in vertebrate equilibration the eye and semicircular canals are sensory control organs, so in photopathy the eye and the body surface may both be sensory control sources for the animal's movements.

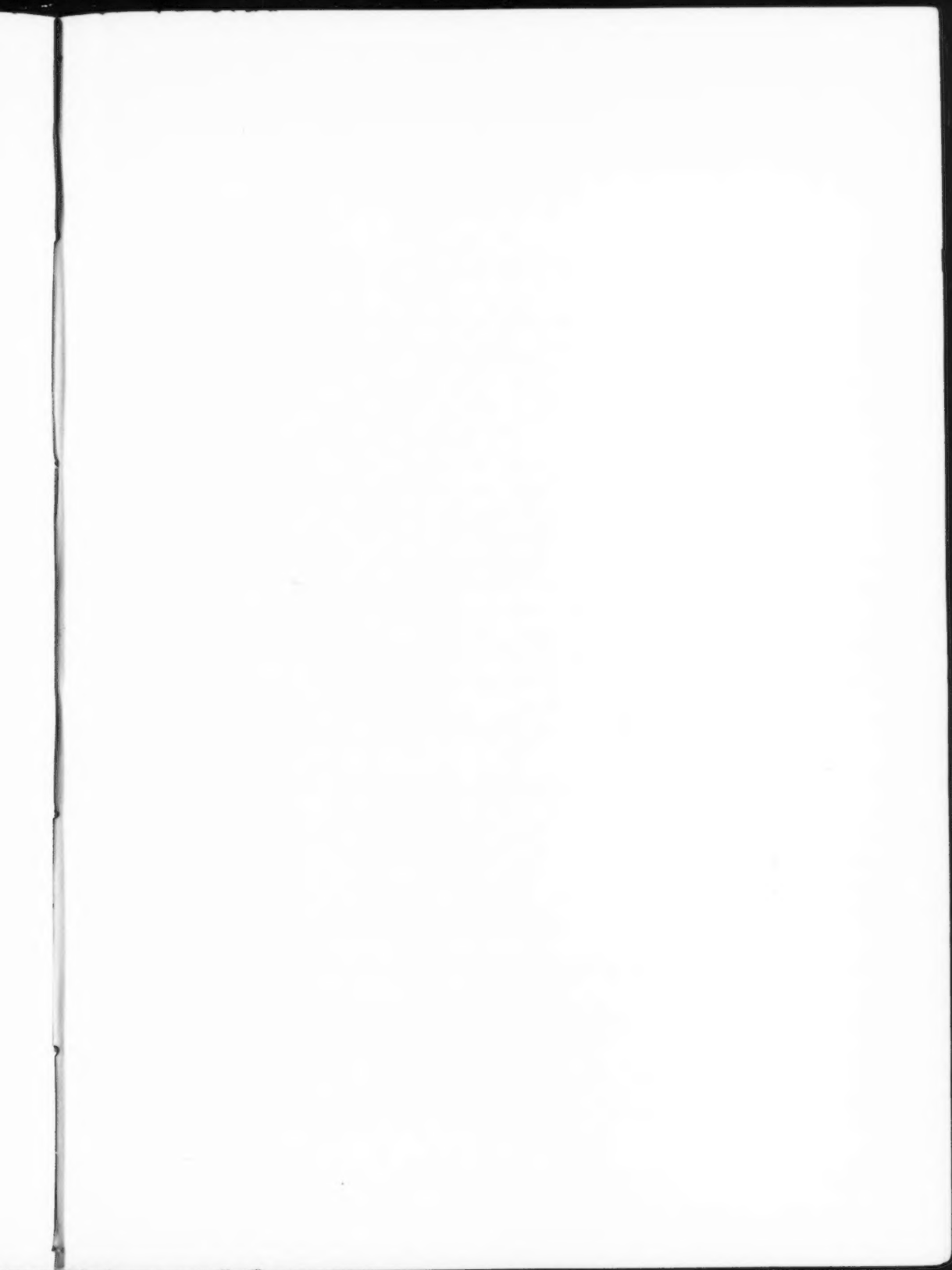
SUMMARY.

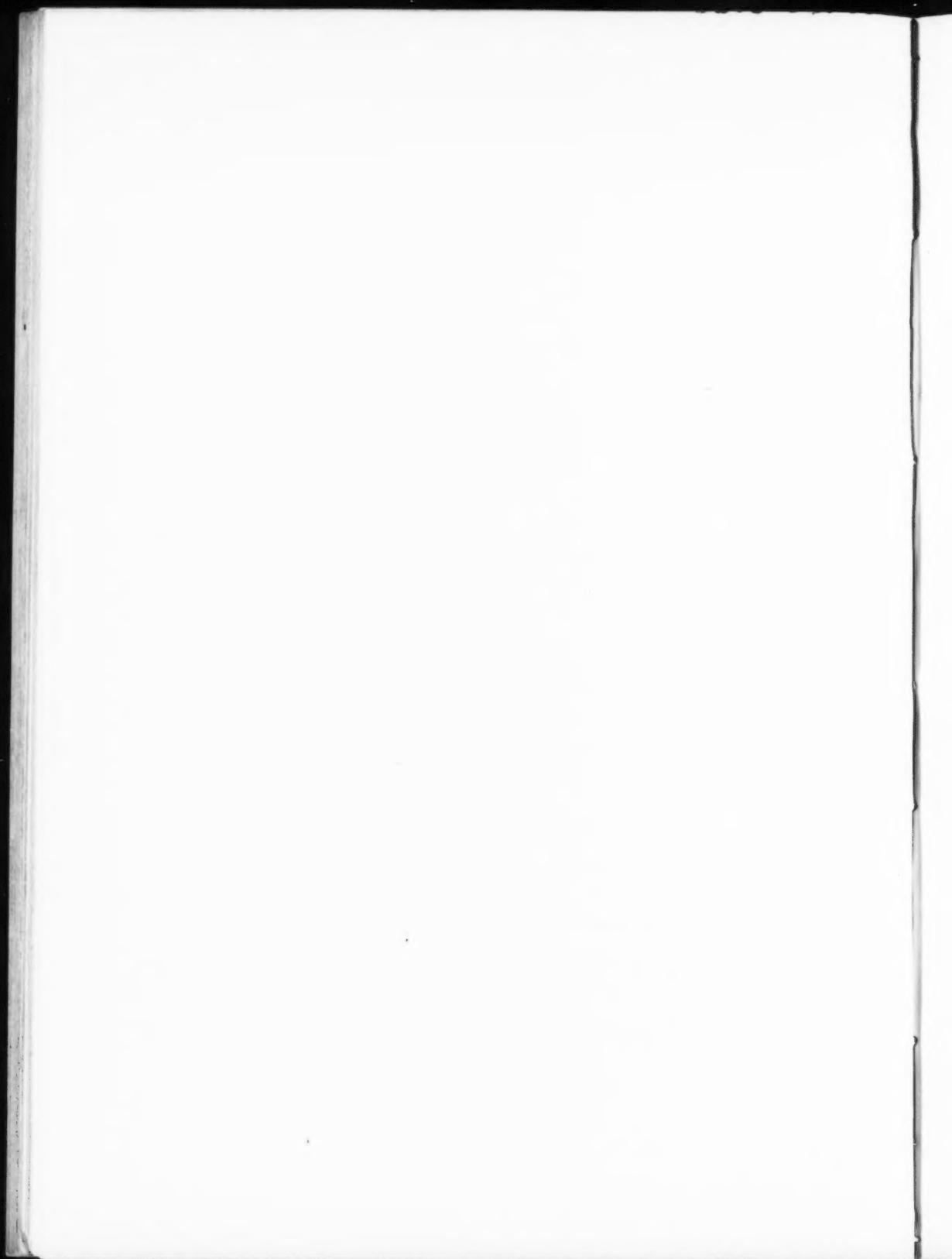
1. *Simocephalus vetulus* Mueller is positively (+) photopathic.
2. The amount of the positive movement varies, within certain limits, directly with the intensity of the light.
3. Diffuse daylight causes a greater positive response than direct sunlight.
4. *Cyclops parvus* Herrick is not photopathic.
5. *Simocephalus* prefers the orange and yellow of a gas spectrum.
6. This response to the spectrum is a photopathic reaction, and is not, so far as is known, chromopathic.

This research was suggested by Dr. C. B. Davenport and was pursued under his direction. Whatever success has been attained is due largely to his interest, aid, and encouragement.

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A MODIFIED SOXHLET APPARATUS FOR THE EXTRACTION OF FAT FROM LIQUIDS.

BY ALONZO ENGLEBERT TAYLOR.

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A CONVENIENT apparatus for the extraction of fats from solutions has been much needed since the investigations of Pfleger and his students have demonstrated that animal tissues must be digested in order to extract their entire fat. The extraction of the digestion products in the ordinary Soxhlet apparatus is a difficult and inaccurate procedure. Commonly the fat is extracted from the digestive solution by shaking it with ether in a glass-stoppered cylinder, the ether being drawn off and the process repeated until the fats have all been secured. Not infrequently, however, on shaking with ether emulsification occurs, a condition difficult to dispose of. Nerking¹ has devised a modification of the older apparatus of Schwarz,² which undoubtedly secures good results but is not easy of manipulation. To the apparatus of Wróblowski³ the same objections apply: it is not in convenient form; it has the disadvantage of a cork attachment; and there is no provision for distributing the ether through the liquid. I believe the following apparatus offers an easy, economical, and complete method of securing the extraction of fat from solutions.

The apparatus consists of three parts: the extractor (Fig. 1); the condenser, with the part connecting it to the plunger; and the reservoir (see Fig. 3). The extractor has the orifice of the siphon tube (*A*) not at the bottom, but at a line below which the tube will hold about 110 c.c. Above this the siphon tube passes up to such a height as to carry off about 70 c.c. at each siphonage. The extractor should be carefully made so that

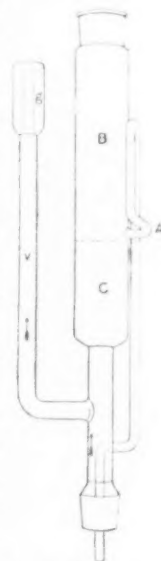


FIGURE 1. The extractor.

¹ NERKING: Arch. f. d. ges. Physiol., 1898, lxxiii, p. 172.

² SCHWARZ: Zeitschrift für anal. Chemie, 1884, xxiii, p. 368.

³ WRÓBLOWSKI: Zeitschrift für anal. Chemie, 1897, xxvi, p. 671.

when the plunger is in place and full of ether, the space (*C*) below the line of the siphon tube (*A*) shall be just large enough to admit 100 c.c. without danger that any of the fluid to be extracted shall be drawn into the reservoir by siphonage.

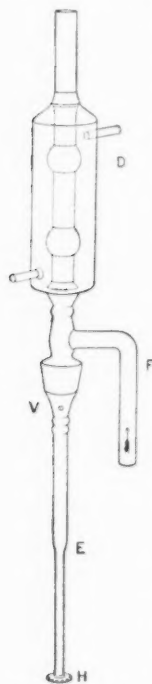
The condenser (Fig. 2, *D*) is of the ordinary form. A worm may be used, as in Fig. 3, if a cooling bath is to be employed. The lower end of the condenser is prolonged into a plunger (Fig. 2, *E*) which passes to the bottom of the cavity of the extractor (Fig. 1, *C*). The plunger has a small opening for an air vent (*I*). A side tube (*F*) carries the ether vapor from the reservoir to the condenser by means of a mercury connection (*G*). The bottom of the plunger is blown in a flat circular form (*H*) and perforated at the edge by means of small openings through which the ether passes out.

The reservoir (Fig. 3, *R*) is simply a glass jar with a ground neck.

There are three connections: (*I*) the reservoir with the extractor; the condenser with the extractor, — both these connections are of ground glass; and the connection (Fig. 3, *G*) for the stream of ether vapor, which is closed by means of a mercury bath.

The working of the apparatus is as follows: The reservoir is placed in a water bath at 60° C. The ether vapor passes up the tube *V*, through the connection at *G*, and into the condenser. On cooling it flows down the plunger (*E*), and at (*H*) is divided into fine streams, which rising pass through the fluid to be extracted and

FIGURE 2. The condenser and the tube.



collect upon it, in the extractor, at (*B*). When the ether has risen to the height of the siphon tube it is siphoned back to the reservoir. Working properly, this apparatus will siphon about every six minutes.

The rapidity and completeness of the extraction depend upon the character of the solution and also upon the instrument. The end of



FIGURE 3. The apparatus ready for use, showing a worm in place of the condenser.

the plunger must be carefully made so as to throw out a stream upon all sides. Milk is quickly and completely extracted. Digested fluids, as in the analysis of meats, are extracted much more slowly, and it is necessary at occasional intervals to loosen the condenser from the extractor, and with the plunger stir the solution in the extractor. If the solution to be extracted be thick and viscid it must be diluted to such a point that the ether can readily pass through it. As the solutions stand, the fats rise to the top and thus are more advantageously presented to the action of the ether. Individual experience will determine how long it is necessary to continue the extraction for different solutions.

The apparatus is blown at least as easily as the ordinary Soxhlet extractor. It is not fragile, is easily adjusted and taken apart, and easily cleaned. No cork connections are used.

One of the chief advantages of such a method is that it permits the analysis of the solution for other substances after the fats are removed. Thus after the extraction of the fats, the glycogen and the nitrogen may be determined, etc. It is an especially good apparatus for the estimation of the fat in milk.

CORRECTION.

Page 137, lines 12 and 13, should read, "the mixture of about 50 per cent $2\frac{1}{2}N$ $Mg\ Cl_2$ with about 50 per cent of sea water."

A NEW FORM OF PISTON RECORDER AND SOME OF THE CHANGES OF THE VOLUME OF THE FINGER WHICH IT RECORDS.

BY WARREN P. LOMBARD AND W. B. PILLSBURY.

[From the Physiological Laboratory of the University of Michigan.]

OF the many forms of apparatus which have been devised for recording small changes in volume of members or organs of the animal body, the most delicate is Ellis's piston recorder.¹ This instrument gave good results in the hands of its ingenious inventor, but was so fragile that it has never come into general use. It consisted of a glass tube, containing a piston of paraffin connected by a straw piston rod with a lever of straw and paper. The piston was turned almost to fit the tube, the intervening space being filled with a volatile oil, such as oil of peppermint or cloves. The fact that the oil tended to dissolve the paraffin was considered an advantage, as it caused a more complete union between them and helped to secure an air-tight closure of the tube. Ellis chose air in preference to water or oil as a medium for transmitting the changes in volume; in part, because air is not liable to the oscillations which are seen in a column of liquid when suddenly moved, and, in part, because air can be employed without subjecting the organ to be studied to external changes in pressure. Since air is easily compressed, it was necessary that his recording apparatus should offer the least possible resistance to the movement of the air; he therefore constructed it of the lightest materials, and sought to avoid every form of friction.

Several investigators² have described more or less modified forms of Ellis's instrument, and Hürthle,³ in his excellent article on transmission of pressures by air, recounts a series of experiments made to ascertain the best form of piston recorder, and its efficiency as compared with the recording tambour. The form which he found to give

¹ ELLIS, F. W.: *Journal of physiology*, 1886, vii, p. 309.

² JOHANSSON and TIGERSTEDT: *Skandinavisches Archiv für Physiologie*, 1889, i, p. 345; SCHÄFER, E. A.: *Journal of physiology*, 1884, v, p. 130.

³ HÜRTHLE: *Archiv f. d. ges. Physiol.*, 1893, liii, p. 301.

the most accurate results, and which he states to be the best instrument thus far devised for recording air waves, was considerably lighter than that used by Johansson and Tigerstedt, the total weight of the movable parts being only 0.68 gram. Although heavier than the form employed by Ellis, it had the advantage of durability.

The writers of this paper, having become interested in a line of work which required an accurate record of very slight changes in volume, found it necessary to employ Ellis's method, and to give to his instrument a more permanent form. Not being at the time aware of Hurthle's results, they made many of the experiments which he describes, and are able to corroborate his conclusions as to the construction of a delicate and accurate piston recorder. The piston must be thin, to minimize the friction in the tube; it must be of small diameter and be connected by a joint with the piston rod and the lever, so that it shall not tilt and bind; the movable parts must be as light as possible, to avoid the effects of inertia and momentum. The instrument to be described meets these requirements. Moreover, the movable parts have but one half the weight of those of Hurthle's apparatus, namely, 0.34 gram. The greatest difficulty to be overcome in devising such an instrument lay in the necessity of having the piston rod connected with the piston by a light ball and socket joint. The piston must move freely in the tube, and yet follow readily the movements of the lever. Not only does the lever describe an arc as it moves up and down, but if it has the flexibility essential to prevent friction on the drum surface, it may readily be displaced a little to one side or the other, so as not to be exactly over the centre of the piston chamber. A simple hinge-joint, such as Jaquet used in his form of piston recorder, is not sufficient to ensure that the piston shall not bind, and, because of the increased friction, fail to respond to delicate changes in volume. This difficulty was overcome by the following device.

A cylinder of plaster of Paris was cast, and then turned on a lathe to fit, but not too closely, a selected glass tube of about four mm. bore, which was to be used as the piston barrel. A depression was then bored in the exact centre of the end of this cylinder to a depth slightly greater than the diameter of the round head of a fine Carlsbad insect pin, no. 2, with a head of a little less than 1 mm. diameter and a shaft $\frac{1}{8}$ mm. thick; this was to be used as the piston rod. From the end of the cylinder, thus prepared, a disk was cut, which was as thin as could be made and not be perforated by the hole

which had been bored to receive the head of the pin. The thickness of the disk was $1\frac{1}{4}$ mm. To connect the pin piston rod by a ball and socket joint with this disk which was to serve as a piston, the disk was put on the table, the pin was placed vertically over it with its head in the hole, (see Fig. 1), a drop of water was placed in the



FIGURE 1. The piston and piston rod slightly enlarged.

hole, and plaster of Paris was added to it, care being taken that the plaster did not spill down on the sides of the disk. When the plaster had set and before it had hardened, the pin, which was still pressed firmly into the hole, was given a rotary motion, so that the head should form a suitable socket for itself, and this movement was repeated from time to time until the plaster had hardened. This piston with its rod, because of its lightness and the presence of the joint,

proved more durable than might have been supposed, and was able to resist much abuse. The one employed by us at present has been in almost daily use for several months. The petroleum oil which was used to lubricate the piston, seemed to harden the plaster.

It has been found that a piston with a ball and socket joint, almost as light and perhaps even stronger than that just described, can be made by turning out a light disk of ivory, hollowing it to form a thin walled cup, perforating the bottom of the cup with a hole the size of the insect pin to be used as the piston rod, bevelling both edges of the hole, and, finally, after putting the pin through the hole so that the head lies in the bottom of the cup, filling the cup with plaster of Paris. In this case, also, as the plaster hardens, the pin should be given a rotary movement and be allowed to form a socket for itself. A little plaster disk of this kind, connected with a light long pin by a ball and socket joint, may find a number of uses; for example, it is a convenient means of connecting a frog's heart with a recording lever.



FIGURE 2. The lever. One half actual size.

The lever of the piston recorder should be very light, in order that it shall not oppose the movements of the piston by its inertia, nor exaggerate them by acquiring momentum. This is absolutely indispensable, for the piston rests on a column of air in the tube, as Ellis says, as on a delicate spring. Further, the compressibility of the medium by which it is propelled, requires that it shall write on the

recording surface with the least possible friction; it must, therefore, be flexible, so as to yield to the inequalities in the recording surface and at the same time be sufficiently springy to maintain a continuous, gentle pressure on the recording surface. Finally, although yielding readily to pressure applied horizontally it must be unyielding in the vertical direction, so that it may give an accurate record of the movements to which it is subjected.

Having these requirements in mind, we constructed the lever as follows. A strip of phosphor-bronze, 0.06 mm. thick, $2\frac{1}{2}$ mm. wide, and 100 mm. long, was shaped at one extremity to form a sharp writing point; the other end of the strip was then bent twice at right angles upon itself, and the pin, which was to serve as the axis of the lever, was passed through holes bored in the shaft of the lever and in the end of the strip which had been bent parallel to it (see Fig. 2). The first bend was 82 mm. from the writing point, the second 11 mm. from the first. The holes for the axis were bored 76 mm. from the writing point, and were just large enough to permit the lever to turn freely on the medium sized pin that served as axis. Another smaller hole was bored through the shaft of the lever, 81 $\frac{1}{2}$ mm. from the axis, to form the bearing of the piston rod. This caused the movements of the piston to be magnified about nine times. The piston rod pin was bent at right angles 12 mm. from the piston, and then passed through the hole which had been prepared for it in the shaft of the lever. It was prevented from falling out by a second sharp upward bend (see Fig. 1).

After the piston rod was attached to the lever, a drop of petroleum oil was put on the plaster of Paris piston, and then a bit of lead, which exactly sufficed to counterbalance the lever and moistened piston, was clamped on the horizontal back plate. The total weight of the counterbalanced lever, with its pin axis, piston rod, and oiled

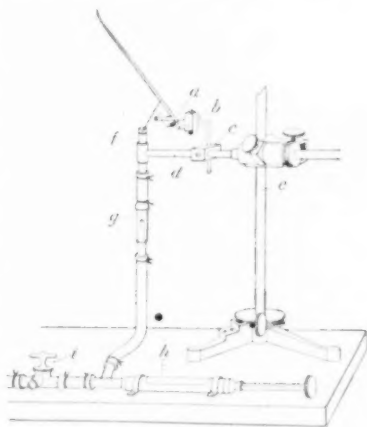


FIGURE 3. The piston recorder and its connections. One fourth the actual size.

piston, was 0.39 gram. A little cone-shaped washer of brass was put on the pin axis, with its apex towards the lever, to keep it from rubbing on the support of the axis.

The pin used as the axis of the lever was supported by being passed through a hole near the extremity of a small horizontal brass rod, (*a*, Fig. 3), and secured by a binding screw in the end of the rod. This rod was in turn supported by a little clamp, (*b*), which fastened it to the horizontal arm of an L-shaped rod, the vertical arm of which was fastened by a clamp, (*c*), to a strong horizontal rod, (*d*), which was clamped near one end to an iron standard, (*e*), and carried at the other end a socket, which held the glass tube which served as the piston barrel, (*f*). By suitable adjustment of these rods and clamps, the bearing of the piston rod could be brought directly over the centre of the glass piston barrel, so that the piston could move with the least possible friction.

The records were taken on a Ludwig kymograph-drum, covered with highly glazed, very thin paper blackened with soot. That the friction of the writing point on the recording surface may be minimized, it is essential that it shall write at a tangent to it, and in a plane parallel to that of the drum. Further, it is very important that there shall be some device for rapidly adjusting the piston recorder, so that the writing point shall barely touch the surface of the drum. Either Boehler's universal stand, Runne's Basel stand, or some similar contrivance will suffice to make this last adjustment quickly at any time during the experiment; the former we found very serviceable.

The piston barrel was connected with the rubber tube leading to the plethysmograph chamber in one of two ways, each of which prevented the oil, which spread from the piston down the wall of the barrel, from entering the rubber tube.

a. A short rubber tube connected the lower end of the piston barrel with a short glass tube, (*g*), into the lower end of which had been sealed the slightly drawn out end of a second short piece of glass tubing. The space around this drawn out end acted as a catch-basin for the oil. The lower end of the second piece of glass tubing connected with the rubber tube which communicated with the plethysmograph chamber.

b. The piston barrel was cemented into one of the short arms of a brass T-tube, which was so placed that the short arms were vertical and the longer stem horizontal. The horizontal stem was clamped

to the standard, and carried a clamp which supported the rods which held the writing lever in place. The end of the lower short arm of the T was corked and acted as the catch-basin for the oil leaking down from the barrel.

The thick walled rubber tube connecting with the piston recorder as described above did not communicate directly with the plethysmograph chamber. It connected first with a short armed T-tube, which communicated, on the one hand, with a syringe, (*h*), and on the other with a three-way stopcock (*i*).

The syringe was air-tight, and it was possible, by movement of the plunger, to alter the amount of air in the system of tubes connecting the recorder with the finger chamber. This is absolutely essential, in order that the changes of volume which are caused by temperature or by movements of the finger may be compensated, and the position of the piston recorder on the drum be adjusted. A large form of hypodermic syringe suffices; but, as very slight movements of the plunger cause large movements of the recording lever, it is best to solder the nut on the rod of the plunger to the syringe, and to move the plunger by screwing it in and out.

The three-way stopcock, (*i*), was connected not only with the piston recorder, but, by means of rubber tubing, with the plethysmograph chamber for the finger. The third opening of the stopcock communicated with the outside air. This arrangement made it possible to connect the piston recorder with the finger chamber, or either piston recorder or finger chamber with outside air. This was necessary, especially at the beginning of an experiment, when the finger was being introduced into the apparatus, and when the temperature of the air in the finger tube was changing rapidly.

Experiments showed that it is important that the temperature of the finger should be kept as far as possible constant, especially during cold weather; moreover, in certain lines of work it is desirable to change the temperature suddenly. Two forms of apparatus were employed for regulating the temperature about the finger. In the first form, the tube containing the finger was surrounded by a jacket through which water of a known constant temperature circulated. The finger tube of this double walled plethysmograph chamber was $11\frac{1}{2}$ mm. long, and $2\frac{1}{2}$ mm. inside diameter; and it was open at one end to receive the finger, and closed at the other by a plate which was perforated by two small brass tubes. One of these tubes was connected by a stiff-walled rubber tube with the three-way stop-

cock, and so with the piston recorder or the outside air; the other tube permitted the introduction of a thermometer into the finger-chamber.

The brass jacket through which the water circulated was a little shorter than the finger tube. The open end of the finger tube projected slightly beyond the jacket, and carried on its outside a flange which enabled the opening between the finger and the tube to be closed by a rubber membrane, sleeve, or glove finger, such as was employed by Mosso in his experiments with the sphygmomanometer. In our experiments, however, the opening between the finger and the brass tube was closed by a cork, as will be described later. A system of reservoirs and tubes permitted the finger to be subjected to a known constant temperature, and allowed the temperature surrounding it to be changed quickly to any desired degree.

The second method permitted the finger to be kept at a constant temperature, but did not allow of sudden changes of temperature. In this method the finger plethysmograph consisted of a glass tube, closed at one end by the finger cork, and at the other by a cork through which a thermometer and the glass tube connecting with the piston recorder passed. A coil of fine wire was wound many times around the glass chamber, and when an electric current was turned on, heated the chamber. The temperature was controlled by means of a rheocord, which permitted the flow of the current to be regulated. During summer weather, the room temperature is sufficiently high and constant for ordinary investigations, and these methods of heating are unnecessary. It is sufficient to enclose the finger in a test tube drawn out at one end for the attachment of the tube connecting with the piston recorder. In all cases it is necessary to protect the subject, the plethysmograph chamber, and the system of air tubes connecting the plethysmograph chamber with the recorder, from sudden changes in temperature. The whole arrangement forms a very delicate recording thermometer, and, if not guarded, may give false results. Ellis speaks of this, and in some of his experiments used a second, control apparatus. We have found that for experiments which last only a short time and are taken with suitable precautions this is unnecessary.

Method of closing the opening between the finger and the reservoir. — One of the greatest sources of error to be encountered in plethysmographic experiments arises from the fact that movements of the arm, be they caused by changes in the tension of the muscles or by some

form of movement of the trunk, as in respiration, tend to cause movements of the finger in the plethysmograph, not always to be distinguished from changes in volume of the finger. As a change of even less than $\frac{1}{2}$ cubic millimetre in volume is recorded by the piston recorder, it is evident that the utmost care must be taken to secure a constant position of the finger as respects the plethysmograph. On account of the mobility of the skin on parts beneath, the arm and hand cannot be fixed absolutely by splints, casts, etc., and if the plethysmograph chamber be fastened to a firm support it is impossible to prevent the finger from moving in the tube. To overcome this difficulty it is necessary to resort to the principle first employed by Chelius, and rediscovered by Mosso, who employed it in his arm plethysmograph, namely, the suspension of the plethysmograph chamber and the arm by a long cord, so that they will move freely and yet together. Even this method is imperfect, in case the space between the finger and the plethysmograph be closed by a rubber membrane or sleeve.

The best way to close the opening between the finger and plethysmograph tube, is to put on the finger a cork ring which exactly fits the plethysmograph tube. Such a ring, if made to fit the finger only moderately tightly, offers considerable resistance to the movement of the finger, and, if smeared with vaseline, readily gives an air-tight closure, without impeding the circulation.

Apparatus for testing the delicacy and accuracy of piston recorder.—

In many of the plethysmographic records taken from the finger not only pronounced pulse curves were obtained, but these curves sometimes showed marked secondary waves, similar to those seen in ordinary sphygmographic tracings. The question of course presented itself, are the dicrotic and other waves shown in the record a true expression of changes in volume of the finger, or are they artificial, *i. e.* caused by vibration of the air or oscillations of the recording apparatus? To decide this question an apparatus was constructed which should mechanically change the volume of the air in the tube connected with the recorder, at about the rate and degree indicated by the pulse waves. A stiff brass lever was fastened firmly to a steel axis, which moved on screw bearings. The lever rested on a specially devised cam which was supported by an axis which carried a pulley driven by an electric motor. On the lever was a short pillar of brass. The pillar projected slightly into the lumen of a somewhat larger glass tube, supported just above it.

The opening of the glass tube was closed by gold beater's skin, kept flexible by being moistened with water. When the cam was revolved, the pillar moved up and down, carrying with it the loose fold of gold beater's skin, and so caused a rhythmical change in volume in the chamber of the glass tube. The upper end of the glass tube was connected by a stiff-walled rubber tube with the piston recorder, and the movements of the air column in the glass tube were recorded by the piston recorder on a kymograph-drum.

In the experimental tests made with this apparatus, to determine the effect of changes in the volume of the air in the tube upon the movement of the piston recorder, the brass lever was sometimes moved by the hand, the end of the lever being held between the thumb and forefinger, and, sometimes, by rotating the cam upon which the lever rested. These tests revealed the following facts. Slow changes in volume, even if extensive, are recorded accurately. Rapid changes in volume, if extensive, may lead to a slight throw of the lever. Changes in volume of the rate and extent of those occurring in the finger, whether caused by vasomotor, respiratory, or pulse movements, are recorded accurately. The question which most interested us, was whether the record of changes in volume as extensive and rapid as those resulting from pulse movements, would show oscillations that might be confused with the dicrotic and other secondary waves which often appear in the pulse curve obtained from the finger. A form of cam was chosen such as would give a sudden upward movement and a slow fall of the brass lever. The changes of volume recorded were somewhat more extensive than those caused by the pulse, and a rate of rotation was readily obtained by the motor which gave a more rapid change of volume than the pulse records showed. To be sure of our facts, we took two records on the same drum, the one of the artificial pulse, obtained from our testing apparatus, and the other, of the normal finger pulse. The pressure of the piston recorder lever was the same in both cases. It was found that changes in volume, which were considerably more rapid and extensive than those caused by the pulse, did not show any throw of the recording lever or any secondary oscillations, and we assured ourselves that the dicrotism and other oscillations which are recorded in the normal pulse curves from the finger, do not originate in the recording mechanism (see Fig. 4). It was found further, that if the cam of the testing apparatus was revolving at a regular rate, and the air in the apparatus was rhythmically carried forwards and backwards

by movements of the adjusting syringe, a combination curve, closely resembling the pulse and respiration curves obtained from the finger, could be produced. This makes it possible to study the effect of respiratory changes in volume upon the shape of the pulse curve, a question of importance.

Latent period of the piston recorder. — This was determined empirically to be but slightly over $\frac{1}{100}$ second, and was found quite constant for both a decrease and an increase in the volume of the air. It was tested by connecting two piston recorders, or a piston recorder and a tambour, writing on the same drum, by a stiff-walled rubber tube one metre in length, a length somewhat greater than that used in our plethysmographic experiments. If the tambour lever or the lever of one of the piston recorders was suddenly moved, the piston recorder under observation was found to start about $\frac{1}{100}$ second late, and to lag at the crest of the curve, at the most, $\frac{1}{10}$ second. This lag depended on the extent and rate of the change

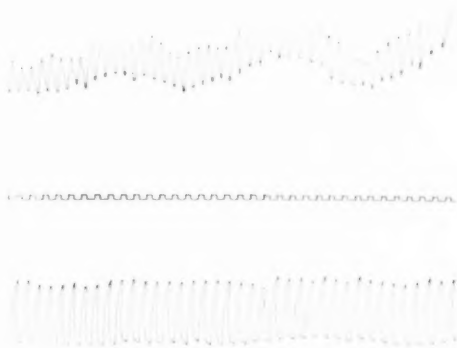


FIGURE 4. One half the original size. The uppermost curve, taken June 5, 1899, on a warm day (room temperature, 29° C.), from the two first phalanges of the left hand. The time, in seconds, is recorded at the bottom of the tracing. The lowest curve, an imitation of a pulse tracing, was obtained by using the apparatus described on page 193.

of volume, and was due to friction of the lever on the drum, compression of the air column, and displacement of the oil about the piston. The displacement of the oil, seen when the movements were extreme and rapid, was only momentary, as capillary force drew the oil back. This error need be feared only when the movements recorded are more rapid and extensive than those which result from ordinary changes in the volume of the finger. The figures here given apply to movements very much more rapid and extensive than those caused by the volume changes of the finger due to the pulse, etc. We concluded from our experiments that such records as are obtainable from the finger, are accurate to within $\frac{1}{100}$ second, if the

lever is adjusted properly. In this connection, however, it is necessary to express again the warning that any undue friction of the lever upon the drum is liable to delay the rate and lessen the extent of the movements of the recording lever.

Calibration of the piston recorder. — As a further test of the delicacy of the apparatus, we measured the change in the volume of the air that corresponded to the movement of the lever. To this end, movements of the lever were compared with the movements of a column of mercury in a tube of small bore, attached to the piston recorder. The volume of the tube used was ascertained first by filling the tube with mercury, and secondly, by weighing the tube when filled with mercury and when empty, and reducing from weight to volume, with suitable corrections for temperature. The results of the two methods agreed very closely. The uniformity of the bore of the tube was established by the fact that the same amount of mercury occupied equal lengths for all parts of the tube; one centimetre of the tube held 9.1 cubic millimetres of mercury. One end of this tube was connected by a rubber tube with the main tube of our apparatus, and to the other end was attached a small piece of rubber tubing filled with mercury and closed by a pinch-cock. The length of the column of mercury in the capillary tube was varied by compressing this short rubber tube between two bits of wood held in the jaws of a burette clamp. The length of the column was read on a scale and compared with the record on the drum. The result showed that a movement of one centimetre in the mercury in the tube would induce a movement of the lever of 5.7 millimetres, *i. e.*, that one millimetre on the drum corresponded to a change in volume of 1.6 cubic millimetres. Furthermore, a change of less than $\frac{1}{2}$ millimetre in the tube, or considerably less than $\frac{1}{2}$ cubic millimetre was found to produce an evident movement of the piston recorder. This also confirmed the previous results, inasmuch as different changes in the length of the column of mercury were always accompanied by relatively equal movements of the lever.

The pulse oscillations recorded in Fig. 4 bear witness to the capacity of our instrument. They were taken on a hot day, when the blood-vessels of the finger were largely dilated. This curve shows also Traube-Hering waves. The dicrotism exhibited was not due to the apparatus. In the artificial pulse shown in Fig. 4 the pulse movements were no less high and steep than those obtained from the finger, but they are free from secondary waves.

Another indication of the delicacy of the piston recorder for changes of slight intensity, was given by the fact that the capillary attraction for water in a tube with a bore of 1 mm. was strong enough to produce marked movements of the lever.

Method of class demonstrations, etc. — It is not difficult to employ the piston recorder in demonstrations before large audiences. If a small silvered cover glass be fastened in the place of the counterbalancing weight on the back of the lever, a beam of light can be thrown on a screen, and the ordinary changes in the volume of the finger caused by the pulse and respirations can be shown. The effect of reflex excitations, such as the application of ice to the skin of the other hand, may also readily be exhibited.

A STATEMENT OF SOME OF THE FACTS OBSERVED WITH
THE PISTON RECORDER.

The account which we have given of the piston recorder was written six months ago, and since that time we have had occasion to make frequent use of the instrument. Our experience has only confirmed our opinion that the instrument, in spite of its apparent delicacy, is very durable, and with proper handling is capable of giving valuable results.

It may be of interest, as showing the value of our method, for us to enumerate some of the more salient physiological facts which have attracted our attention during our preliminary tests of this apparatus.

All of the tests were made with only two phalanges of the first or second finger of the left hand. When one of these was inserted into the plethysmograph chamber, the heat of the finger caused a rapid expansion of the air in the chamber and the system of tubes connecting it with the recorder. A considerable time was required for an equilibrium to be established between the heat of the finger and the temperature of the surrounding air. During this period of adjustment, the piston lever was kept level, either by gradually drawing out the piston of the adjusting syringe, and so enlarging the space, or by turning the three-way stopcock so as to give free communication with the outside air. As the constant rise of the piston lever due to the expansion of the air lessened, the fluctuations of the volume of the finger became evident. These volume changes were manifested by quick up-and-down movements of the lever due to the pulse, slower rhythmical movements which correspond to respiratory

movements, and much slower variations, presumably produced by vasomotor changes.

The extent of all of these fluctuations was very markedly influenced by the temperature to which the body as a whole, and more especially the finger under observation, was exposed. If the room was cold, the volume changes described were hardly perceptible, while in a warm room all three of these changes showed themselves. The difference between the size of the variations in summer and winter was very great. The general effect of the room temperature on the body could be counteracted in part by changing the temperature of

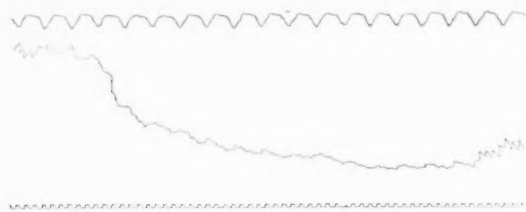


FIGURE 5. One third the original size. Plethysmographic record from two phalanges of middle finger of left hand, May 1, 1899. Height of piston lever, when horizontal, above bottom of seconds tracing = 34 mm.; height of respiration lever, when horizontal, above bottom of seconds tracing = 75 mm.; length of piston lever, 70 mm.; length of respiration lever, 93 mm. Respiration lever wrote 0.8 mm. to the right of the piston lever. Subject stated at the close of experiment that he had caught himself thinking actively of his work, and had tried to stop thinking.

the finger. When the temperature of the room was low, and the fluctuations were barely visible, it was possible to carry on experiments by raising the temperature of the air in the finger chamber to about 34°C . On the other hand, it was found that changes in volume that were fairly well marked in a

moderately warm room, would become greatly lessened, or entirely disappear if the temperature of the air about the finger were lowered to about 20°C . We can confirm the results of Mosso, François-Franck, Sewall, Sanford, and others, that cold applied to the other hand noticeably decreases the volume of the finger under observation. Thus when the right hand was dipped into cold water there followed a marked shrinkage of a finger of the left hand, and the extent of the oscillations of volume due to the pulse, respiration, etc., greatly decreased.

Very similar results accompanied the pain caused by stimulating the skin of the other hand with an induced current. Two to three seconds after the stimulus was applied, there was a marked shrinkage of the finger, and the height of the pulse oscillations lessened.

These effects seemed to be common to nearly all the psychical and reflex disturbances that were noticed. The odor of a stale cigar held under the nose, pulling a hair, blowing in the face, and other minor persecutions, invariably gave the same result. During the progress of the experiments, the mind of the subject would very frequently drift to some problem of daily routine or one connected with the experiment. At such times the volume of the finger would show a marked fall (see Fig. 5). The only two exceptions to this rule were both at times when the mental state was one of amusement; once at a remembered event, the other time, over an incongruous conjunction of ideas in the train of thought. In these two cases a well marked and long continued rise was observed.

Sudden changes in respiration produced a very pronounced variation in the ordinary course of the experiments. A partly suppressed yawn, followed by a long expiration, for instance, was accompanied by a fall in the height of the lever. The effects of Valsalva's and Muller's experiments to cause a weakening or temporary cessation of the pulse, were, of course, at once evident.

In addition to recording changes in the circulation caused by forced respiratory movements, the instrument gave a striking picture of the normal variations in the volume of the hand when the subject was at rest. By recording the respiration with Marey's pneumograph, just over the piston recorder tracing, it was possible to notice the time relations of the movements of the wall of the chest or abdomen and the corresponding oscillations of the volume of the finger. These changes of volume were seen to hold about the same relation to the breathing movements, as has been observed for respiratory fluctuations of the blood pressure. The volume begins to increase at about the middle of inspiration, and continues to rise until about the middle of expiration; it then falls during the latter part of expiration and the beginning of inspiration. This general correspondence is, however, subject to wide fluctuations. The effects of respiration were usually shown when the volume of the finger was large. No constant relation, however, was seen to exist between the size of the pulse and the respiratory oscillations. Sometimes the pulse oscillation would be fairly large when no respiratory change was to be seen, and, at other times, there would be well marked respiratory oscillations when the pulse movements were scarcely visible.

The height of the pulse wave was very plainly influenced by the

volume of the finger. By large volumes the oscillations were relatively small, by medium they were greatest, while by small volumes they were very small or altogether disappeared. As the volume changed from large to medium, the downward oscillations were the first to increase in size and *vice versa*.

In the pulse wave the dicrotism quickly attracts attention. The dicrotism was seen to undergo marked variations, and our method bids fair to contribute important facts as to the origin of the secondary waves and the influences affecting them. A propos of the discussion as to the origin of the dicrotic wave, it is worthy of note that this wave is clearly to be seen in experiments, such as ours, which deal with the phenomena of the extreme peripheral circulation.

In addition to the pulse and respiratory changes in the volume of the finger which have been described, still another change, having a much longer period, was observed. The duration of these longer waves was from seven to ten seconds from crest to crest, which makes it probable that they were of the same type as the Traube-Hering blood pressure waves.

Still another series of facts exhibited by our tracings relate to the periodic changes in the rate of the heart. The pulse rate was found to vary with the respiration. It was higher during the latter part of expiration, and lower during inspiration. Moreover, in some cases, when the breathing was suspended, this rhythmic change in the beat of the heart showed a tendency to persist. Still another rhythmic change in the rate of the pulse, recurring in longer periods, was noticed at times.

The facts which we have here stated may suffice to indicate the value of the piston recorder, not only as a means of demonstrating many important circulatory phenomena but as giving to the plethysmographic method an added delicacy.

SECONDARY RHYTHMS OF THE NORMAL HUMAN HEART.

By WARREN P. LOMBARD AND W. B. PILLSBURY.

[From the Physiological Laboratory of the University of Michigan.]

THE rate of the beat of the normal human heart, when the subject is at rest, undergoes two forms of periodic variation. One of these changes of rate is associated with the action of the respiratory centre, and may be called the respiratory rhythm; the other corresponds to changes in the volume of the finger attributable to vasomotor activities, and may be termed the vasomotor rhythm. These statements are based on experiments made on five men from 21 to 50 years of age. The respiratory rhythm was observed on all the men, but the vasomotor changes were found on but four, from 21 to 43 years of age, the other man not having been examined with reference to this point.

The heart beat may therefore be said to have a primary rhythm which originates in the heart muscle, and two secondary rhythms which are associated with the activities of the respiratory and vasomotor nervous mechanisms respectively.

In the course of experiments in which changes in the volume of the finger were recorded by the piston-recorder plethysmograph, it was observed that the heart beats were quickened during inspiration and slowed during expiration. Although this respiratory rhythm of the heart was more pronounced when the respiratory movements were labored, it was seen during quiet, shallow respirations, and, what is still more remarkable, it often continued when respiratory movements were apparently entirely suspended, either voluntarily, or as a result of painful excitations of the skin. Just after these observations had been made, the laboratory was so fortunate as to be visited by Professor A. Mosso of Turin. At his suggestion we sought to verify our observations by comparing the pulse of the carotid with the respirations. With his kind assistance this was done, and a rhythmic change of the heart rate during quiet respirations was again observed.

A study of the literature has shown our results to be in line with the observations of others. The respiratory change in the heart rhythm has been, however, generally regarded as a result of more or less forced or modified respiratory movements, rather than as a constant accompaniment of quiet respiration.

Sommerbrodt,¹ following a cue given by Hering, to be cited later, found that the heart rate was changed as an after effect of the Valsalva experiment, by breathing compressed air, and by the forced expiration required in reading and singing. Fredericq² noted that during very slow respirations, one could detect with the finger a difference in the heart rate between inspiration and expiration, amounting to two beats (five in expiration and seven in inspiration). Cushney³ noticed recently a marked prolongation of the beat from $\frac{3}{4}$ to $\frac{1}{2}$ of a second, upon a deep breath. Binet and Courtier⁴ describe changes in the heart rate which accompany quiet respiratory movements, and on page 119 state that these changes in the heart rate are an important if not the chief cause of the respiratory oscillations in the volume of the hand. We shall later have occasion to refer to the results which have been obtained from the study of animals.

After we had satisfied ourselves that the respiratory rhythm of the human heart is a normal, constant phenomenon during quiet respiration, we proceeded to make further experiments to ascertain the correctness of our observation that the respiratory rhythm of the heart may continue after respiratory movements have stopped. It was in the course of these experiments, that we noticed that the heart rate undergoes another form of periodic variation, at intervals of from 8 to 15 seconds. The length of these periods was suggestive of a vasomotor influence, the time being about that of Traube-Hering blood pressure waves. This idea that the heart might be influenced either directly from the vasomotor centre, or indirectly, as a result of vasomotor effects, was confirmed by an experiment to be described later, in which we found that the slow rhythmic change of the heart rate corresponds very closely to changes in the volume of the finger attributable to vasomotor action. We have called this long periodic variation in the heart rate the vasomotor rhythm of the heart.

¹ SOMMERBRODT: *Archiv für klinische Medizin*, 1881, i, p. 60.

² FREDERICQ: *Archives de biologie*, 1882, iii, p. 55.

³ CUSHNEY: *Journal of experimental medicine*, 1899, iv, pp. 3-4.

⁴ BINET and COURTIER: *L'année psychologique*, Paris, 1896, p. 87.

METHOD.

Before giving a more detailed account of the facts which we have observed, we ought to describe briefly the method employed. The radial pulse was recorded by Marey's sphygmograph *à transmission*, the respirations by Marey's pneumograph, and the changes in the volume of the finger by a piston-recorder plethysmograph.¹ When the records were taken on slow drums, the time was recorded in seconds by a chronograph connected with an interrupting clock, and when quick moving drums were used, a fork making fifty double vibrations per second wrote the time directly. All records were taken on highly glazed, blackened paper. To obtain records extending over considerable periods of time and yet have the recording surface moving rapidly, loops of paper three metres long were prepared, and were stretched between two upright drums, one of which, the driving drum, was on a Baltzar kymograph. In some of the experiments, the volume changes in the finger were recorded on a quick drum, in others, the sphygmogram from the carotid or radial was written on a quick drum, and in still others the volume-changes of the finger of one hand were recorded on a slow drum, and, at the same time, the radial pulse of the other hand was recorded on a quickly moving surface. In all the experiments, a record of the respiratory movements of the chest or abdomen was taken.

In experiments in which records were taken simultaneously on two drums, a Morse key was put in circuit with two electric signals, arranged to write, the one on the slow drum, the other on the fast drum. The key was closed from time to time during the experiments, and the corresponding parts of the two records were marked by the signals.

In all the experiments, the temperature of the room was kept nearly constant, and the subject was protected as far as possible from all forms of disturbing psychic influences. When anything likely to disturb or excite the subject was noticed, the Morse key referred to was closed and the time of the occurrence was marked by the signals on the recording surfaces.

A word may be said as to the way we studied our curves. In the early experiments the time was marked in seconds on the curve, and

¹ The instrument is described by us in this number of the Journal.

this record assuring us that the drum maintained a regular movement, we read the pulse and respiration lengths in millimetres by the aid of Jacquet's curve analyzer. Of course, before the reading was taken, correction was made on the curve for any displacement caused by the fact that the recording levers wrote in an arc. In all the later experiments with quick drums, the time was written in fiftieths of a second. In reading these records, the fiftieths were first marked off in fives, then in twenty-fives, and finally in hundreds and thousands. In one of the longer experiments more than fifty-six hundred tuning fork vibrations had to be counted. The beginning of each pulse and respiration movement was then connected with the time record by a perpendicular line, and finally the time relations of these processes were recorded in tabular form. The facts contained in the tables thus constructed did not reveal themselves clearly, however, until the numbers were plotted in chart form. These charts were made on cross section paper. The time was laid off on the abscissa in fiftieths of a second to the centimetre. At the proper points, perpendiculars were erected to mark the time of the beginning of each inspiratory and each expiratory movement. The duration of the successive pulse movements was then plotted on the chart. The ordinates for this curve of heart rate measured the duration of the successive heart cycles in fiftieths of a second; each centimetre of the ordinates corresponded to one-fiftieth of a second. Each pulse length (heart cycle) was represented by a dot, placed above a point on the abscissa corresponding to the beginning of the pulse movement, and at a height corresponding to the duration of the heart cycle as measured by the ordinate. These dots were then connected by straight lines. The curve thus obtained was seen to fall when the heart quickened, and to rise when it slowed, and the relation of this change of rate to the respiratory movements was strikingly shown. In some of the charts changes in the volume of the finger also were plotted. Dots were placed above the points on the abscissa corresponding to the times that the volume of the finger was large or small. The extent of the change of volume was not recorded, and all the dots representing large volumes were placed on the same level and those representing small volumes were placed at a level a short distance below. The dots were then connected by lines, and a curve was obtained which showed the time relations of the volume changes of the finger as compared with the respiratory movements and the changes in heart rate.

RELATIVE EFFECT OF RESPIRATORY AND VASOMOTOR
INFLUENCES ON RATE OF HEART.

When studying the respiration rhythm of the heart, we were at first puzzled by irregularities which showed themselves even when the subject was at rest and apparently free from all sensory or psychic disturbances. These irregularities are for the most part readily explained by the fact that the heart rate is being all the time more or less influenced by the effects, not only of respiratory, but of vasomotor activities. Although these influences appear to be always at work, either one may be very slight, even when the other is quite effective. This is especially true of the vasomotor action. The respiratory influence is nearly always observable, while the vasomotor effect can at times be detected only by slight irregularities in the respiratory rhythm. Apparently the strength of either influence may alter independently of the other, but, as a rule, a change of intensity of the one is accompanied by a corresponding change in intensity of the other. The extent to which the rate of the heart shall be altered at any given time by respiratory and vasomotor influences depends, in part, on the strength of these influences, and, in part, on whether at the time in question they are acting together to slow or to quicken the rate, or are exerting antagonistic effects.

When the vasomotor effect is almost absent, quiet respirations may cause the time elapsing between the succeeding heart beats to vary only one or two fiftieths of a second, while stronger or more prolonged respirations may alter the interval between the succeeding beats three or four fiftieths of a second. When the vasomotor effects are at all marked, the influence of quiet respirations may be favored and hence be made to appear unusually large, or may be opposed and greatly lessened, stopped, or even made to appear to act in a direction the opposite to that ordinarily seen during inspirations and expirations. When both vasomotor and respiratory processes and their accelerating and slowing effects are acting antagonistically, each may be seen to lessen the effect of the other to some extent; the respiratory effect in extreme cases may be wholly prevented, but the vasomotor effect is only temporarily interrupted. When like phases of the two influences are acting simultaneously, very marked changes in the heart rate may occur within short intervals of time. Thus, in one of our curves, it was found that after the breathing had been suspended, without straining, the intervals between the heart beats changed in five seconds from thirty-two to fifty-eight fiftieths

of a second, which is equivalent to a change in the rate of the heart from ninety-four to fifty-eight beats per minute.

THE RESPIRATORY RHYTHM OF THE HEART.

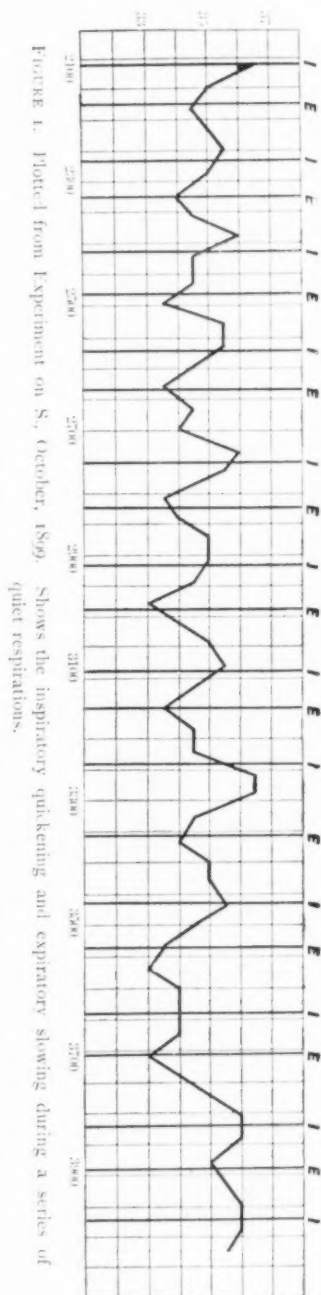
The facts just cited make it evident, that if one would see the respiratory rhythm of the heart during quiet respiration to advantage, he should examine the heart rate during an experiment when vasomotor and other influences affecting the rate are minimal. Such an opportunity was found in the following experiment.

The subject was a healthy man of twenty-one years of age who was accustomed to making psychological experiments upon himself, and so had himself under control. In the present experiment he sat with closed eyes, and the plethysmographic records, taken simultaneously with the sphygmographic records, showed that vasomotor changes were small and that psychic disturbances were few. The pneumograph records indicated that the respirations were of medium depth and fairly regular. The numbers in the following table state in fiftieths of a second the duration of the succeeding heart cycles. They are arranged in the order in which they occurred, and in two groups, according as they took place during the inspiratory or expiratory movements of the chest.

TABLE I.
Experiment on S., Oct. 9, 1899.

Heart Cycles during Inspiration.		Heart Cycles during Expiration.			
35.5	35.0	34.5	35.0	35.5	
35.0	34.0	34.5	36.0		
34.5	34.5	33.5	35.5	35.5	
34.5	33.5	34.5	34.0	34.0	36.0
35.5	33.5	34.0	35.0	35.0	
34.5	33.0	34.0	35.0	35.5	
34.5	33.5	34.5	34.5	36.5	36.5
34.5	34.0	35.0	35.0	35.5	
34.5	33.5	33.0	34.0	34.0	
34.0	33.0	34.0	35.0	36.0	
36.0	35.0	35.5	34.0	34.0	35.0

Only a portion of the experiment is given in the table, and this was selected from the part where the breathing was most regular and the respiratory effects on the heart were most typical. Even here irregularities show themselves. It is often hard to tell from the pneumograph record, the exact moment that an inspiratory or expiratory movement ceases and the next phase begins; moreover, even when the subject is quiet, vasomotor or psychic influences may affect the heart rate slightly. In spite of minor irregularities, the table shows that the first pulse following the beginning of inspiration is generally shorter than the last pulse during the preceding expiration; that the second pulse during inspiration is generally shorter than the first; that the first pulse beat after the beginning of expiration is generally longer than that at the close of the preceding inspiration; and, finally, that each succeeding pulse during expiration tends to be longer than the pulse before it. The acceleration of the heart during quiet inspiration and the retardation during quiet expiration is to be seen to better advantage in Fig. 1, which was constructed from the results obtained from the same part of this experiment. The abscissa gives the time of the beginning of the inspirations, expirations, and heart beats, in fiftieths of a second, and the ordinates, the duration of the successive heart cycles, in fiftieths of a second. A rise of the heart curve indicates a slowing, and a fall of the curve a quickening of the heart.



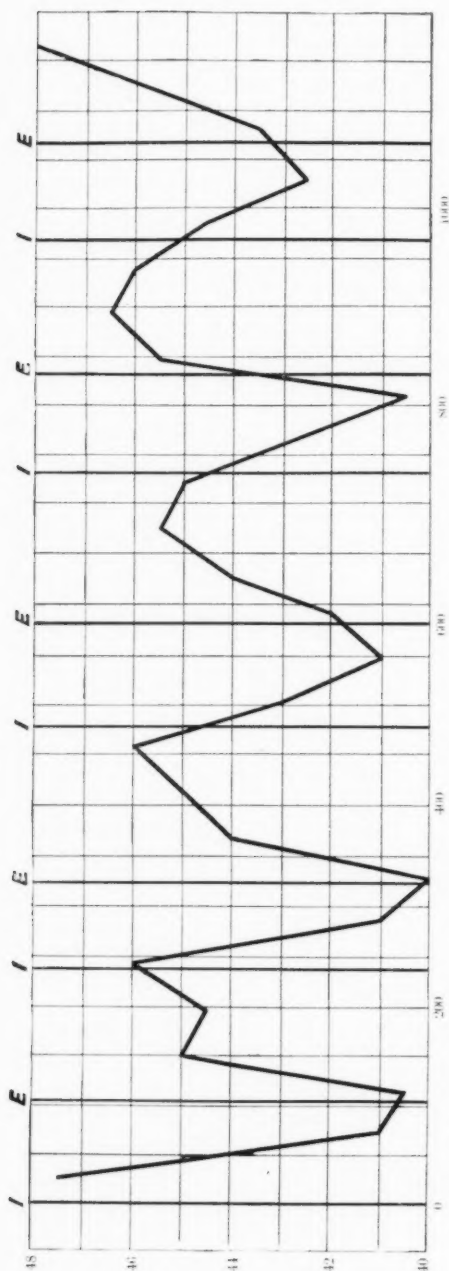


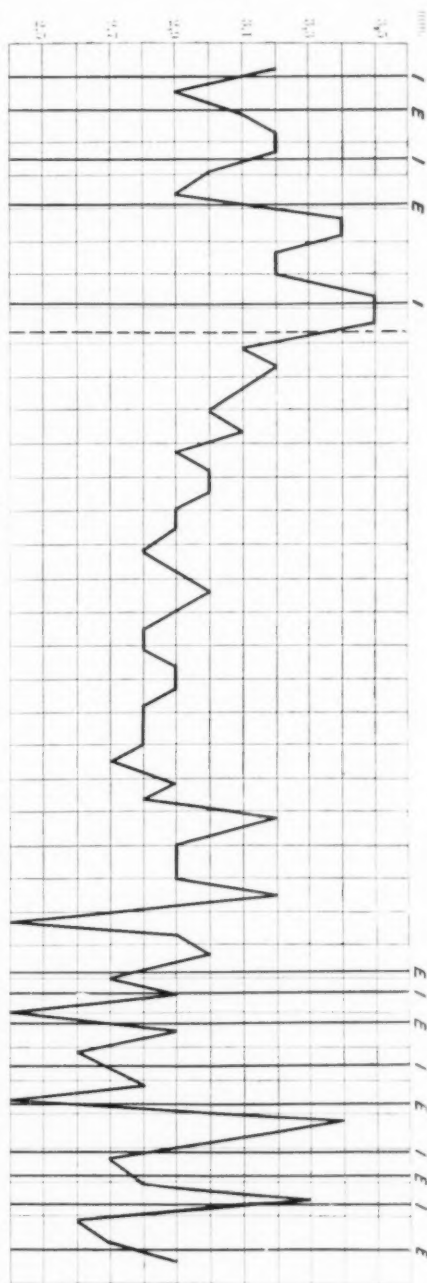
FIGURE 2. From experiments on L., August, 1899. Illustrates respiratory rhythm of the heart when the breathing is long and deep.

The respiratory rhythm of the heart generally becomes more accentuated when the respiratory movements are prolonged and deepened. This is illustrated in Fig. 2 (see also last half of Fig. 5), which is a graphic representation of the results observed in an experiment on L., in August, 1899. The results were plotted in the same way and on the same scale as in Fig. 1. Although voluntary increase of the respiratory movements is often, as in this case, accompanied by a more pronounced respiratory rhythm of the heart, such is by no means the invariable result. On the contrary, any marked change in the respiratory movements produced voluntarily is likely to disturb the normal relation between the heart rhythm and the movements of the chest wall. Fig. 3 gives the alterations in the rhythm of the heart in relation to the respiratory movements, as they occurred in an experiment on L. April 30, 1899. The figure differs from the

preceding in its construction, in that the time relations are recorded differently. Every 8 millimetres on the abscissa equals 4 millimetres of the curve and 1 second of time, and every centimetre of the ordinate equals 0.1 millimetre of the curve and $\frac{1}{40}$ second.

In this experiment, after a period during which the respirations were of the ordinary quiet type, the breath was held in inspiration for about 26 seconds. There was no straining, and the pneumograph curve shows no oscillations during the period. Only the first two respirations which occurred when the breathing was resumed were very deep, the third was but little more than ordinary, and the rest were of the same size as those before the breath was held. A glance at Fig. 3 shows that during the first two respirations which are pictured, the heart quickened in inspiration and slowed in expiration, but that during the expiratory movement which just

FIGURE 3. Experiment on L., April, 1899. An example of the persistence of respiratory rhythm after respiration has been voluntarily suspended and of the fact that the heart rhythm and breathing movements may not be synchronous for some time after breathing is resumed.



preceded the inspiration that was held, the usual slowing was followed by a quickening, and this by a slowing which was continued into the period of inspiration. We regard this disagreement between the heart rhythm and the respiratory movement as a psychic effect accompanying the preparation for holding the breath. Examination of the pneumograph curve in the original tracing shows that this expiratory movement was not carried evenly through, but was interrupted midway and then continued. The interruption did not take the form of an inspiratory movement, however, and the impression which the curve gives is that the ordinary respiratory movements were inhibited, inspiration being prevented. It looks as if the subordinate respiratory mechanisms had received orders at the same time from two different sources, from the respiration centre and from the cerebrum, and that after slight hesitation they had obeyed the will. The fact that the respiratory rhythm of the heart persisted, and that during the expiratory movement of the chest an inspiratory quickening of the heart rate occurred, is in favor of the view that the voluntary impulse only partially inhibited the activity of the respiratory centre. Many phenomena of a similar type have been observed in the course of our work.

After the respiratory movements had ceased, the rate of the heart continued to vary more or less rhythmically. The extent of the variations was less for a time than those which occur during ordinary respiration, which suggests that the respiratory centre yielded somewhat to the inhibiting voluntary impulse. Toward the end of the period of suspended respiration, however, the rhythmic changes in the heart began to be very marked, which suggests that the dyspnoëic condition which was developing was exciting the respiratory centre to increased activity.

When the respirations were resumed, the heart rhythm and respiratory movements showed some divergencies. It may have been that the will was still acting on the respiratory mechanism.

Such an experiment reveals several facts: it shows that the respiratory rhythm of the heart may vary independently of respiratory movements; that it may persist after the respiratory movements have ceased; and that although the variations in the heart rhythm may at first lessen, when the respirations are voluntarily suspended, if the breath be held for long, they may increase beyond what is seen during ordinary quiet breathing.

Fig. 4 exhibits another case in which the breath was held, this time for only a short period. This illustration was obtained from an experiment on P., May 15, 1899. In this case only one respiration was omitted; the heart rhythm went on unchanged in spite of the omission.

The only reference which we have found to any observation of this kind, is a brief footnote in an article by Binet and Courtier,¹ in which the writers state that when the respirations are voluntarily prolonged, the heart may show two or three periodic changes of rate for each respiratory movement, and they say that during these supplementary

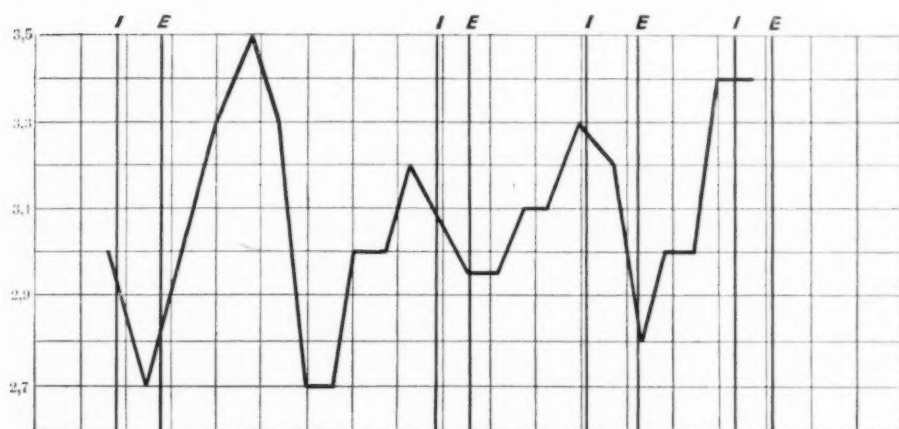


FIGURE 4. Drawn from curve on P., May, 1899. Shows that respiratory movements may persist when the breath is held.

periods the two phases of acceleration and retardation are less accentuated than in periods corresponding to normal respirations. We have failed to find in their article any explanation of the changes in the heart rhythm which they have noticed.

Fig. 5 supplies another illustration of the fact that the heart during modified respiratory movements may exhibit a respiratory rhythm which differs in time from the movements of the chest. The experiment which this chart represents, was made on P., Aug. 1, 1899. In this chart, as in Figs. 1 and 2, the time relations are shown in fiftieths of a second. The subject of the experiment had been breathing quietly up to the time that the drum was started: he then gave a series of short, shallow, panting movements, and followed these by pro-

¹ BINET and COURTIER: *L'année psychologique*, Paris, 1896, p. 124.

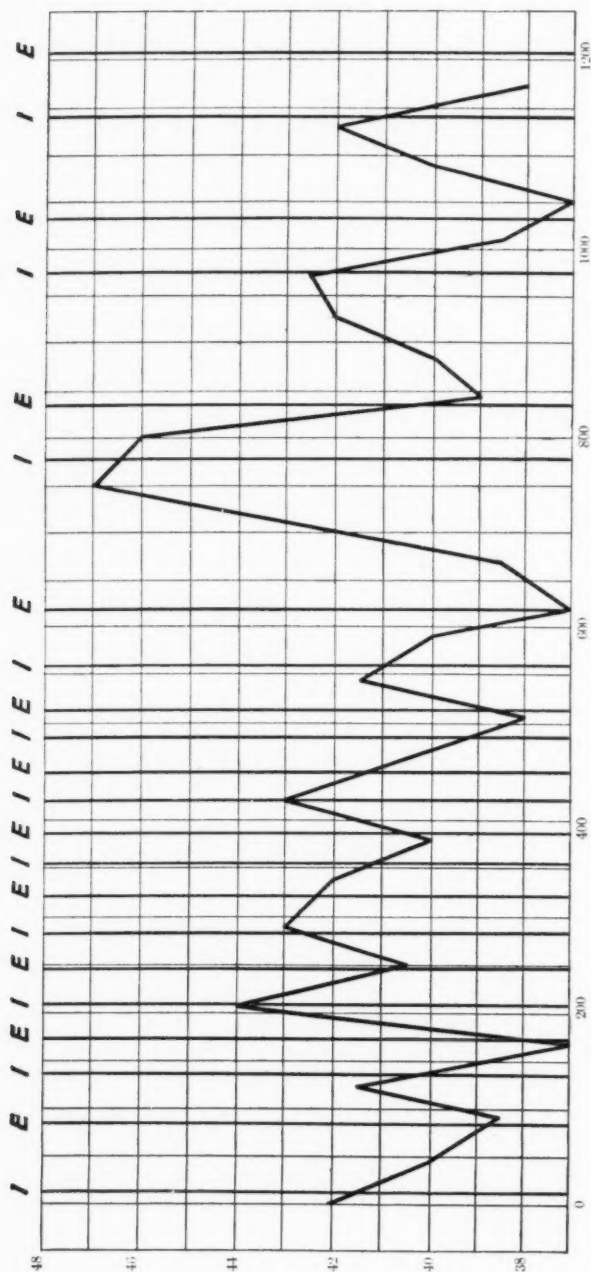


FIGURE 5. Plotted from curve taken on P., August, 1899. The respiratory heart rhythm has a rate different from that of the chest wall when the latter becomes very rapid, although it at first tends to follow the increased rate of the breathing movements.

longed and somewhat deepened respirations. The heart rhythm, which at the beginning of the experiment was in harmony with the respiratory movements, at first quickened as the respiratory movements quickened, but soon returned to about its former rate, so that toward the last of the panting movements the heart rhythm was only about half as fast as the movements of the chest. When the panting movements ceased, the respirations became longer and deeper than they were at the beginning of the experiment, and the heart rhythm changed in a corresponding manner.

The facts which are revealed in these charts have all been seen by the writers in other experiments. They all go to show that although under ordinary circumstances the heart rhythm and the respiratory movements of the chest tend to keep step, this relation is not invariable. If the respiratory movements are voluntarily modified, whether much slowed, stopped, or greatly quickened, the respiratory rhythm of the heart may persist, or may alter independently of the rate at which respiratory muscular mechanisms are made to act.

The first experiments upon the relation between respiration and heart rate were made by Einbrodt,¹ at the suggestion of Ludwig. He ascertained two important facts, namely: that the rate of the heart may be made to change by altering the pressure of the air in the lungs, and that this change fails to occur in case the vagi nerves have been cut. Some years later Hering² repeated and extended these experiments. He found that changes in pressure artificially produced in the lung, when the thorax was open, altered the heart rate if the vagi were intact, but failed to do so if they were cut. He concluded that the normal respiratory changes in the heart rate are due to reflex stimuli from the lung which in inspiration inhibit the action of the vagus centre. Marey's³ view that the vagus is mechanically stimulated by the respiratory movements of the lung was disproved by Hering's work. Sanderson's⁴ idea that the change of rate is an effect of carbon dioxide in the blood may be lightly passed over as not in accord with our present knowledge of the condition of the blood during different phases of respiration. Fredericq⁵ found that

¹ EINBRODT: Sitzungsberichte der königl. Akademie der Wissenschaften zu Wien, 1860, xl, Ab. 2, p. 361.

HERING: *Ibid.*, 1871, lxiv, Ab. 2, p. 333.

² MAREY: *Circulation du sang*, 1881, p. 462.

⁴ SANDERSON: Croonian lecture, March 7, 1867.

⁵ FREDERICQ: *Archives de biologie*, 1882, iii, pp. 80 ff.

respiratory movements of the chest wall may be accompanied by changes in the heart rate after enough of the chest wall has been cut away to prevent respiratory movements of the lung. He concluded that Hering's theory, that the vagus centre is reflexly stimulated from the lung, is therefore untenable and that the rhythm of the heart must be caused by an action of the respiratory centre on the vagus centre. Wertheimer and Meyer¹ found that inspiratory quickening of the heart rate in man is wholly prevented by the administration of atropin. They also observed on dogs that if the cord was cut just below the medulla, so that all chest movements ceased, the respiratory movements of the head and the respiratory rhythm of the heart persisted, which clearly demonstrated the central origin of the variations in the rate of the heart.

Our results are in harmony with those of the later experimenters. The fact that the respiratory rhythm of the heart may continue in man when respiratory movements of the chest and abdomen have been voluntarily inhibited, and that the rate of the heart rhythm may persist almost unchanged when the chest is made to perform very slow respiratory movements, or to make very rapid panting movements, shows that the changes we observed in the rate of the heart were not dependent on physical changes occurring within the chest or abdomen, or on reflex stimuli which might be generated as a result of changes in the air pressure in the lung, but were caused by a spread of impulses from the respiratory centre upon the heart centre.

If this view be correct, our experiments show that the respiratory centre may maintain its rhythmic activity after external respiratory movements have been voluntarily inhibited, and also while they are being voluntarily made to occur at a different rate. From this it would appear that the voluntary control of the respiratory muscles during talking, singing, etc., is accomplished not so much by a change in the activities of the respiratory centre as by influencing the subordinate respiratory mechanisms in the spinal cord. This view is not, however, opposed to the idea that the respiratory centre may be more or less influenced by volition, for in our experiments, when the respiration was suspended (without straining) we have seen the respiratory rhythm of the heart to be feebler than when normal respirations were in progress. Moreover, a voluntary slowing or quickening, flattening or deepening, of the respiratory movements may within limits be accompanied by corresponding changes in the respiratory

¹ WERTHEIMER and MEYER: *Archives de physiologie*, 1889, p. 24.

rhythm of the heart. In making these statements we do not mean to imply that the respiratory centre may not be influenced by sensory stimuli from the lung.

VASOMOTOR RHYTHMS OF THE HEART.

Comparatively late in our work on the respiratory rhythm of the heart, we noticed in one of our charts a wave that lasted longer than those attributable to respiration and that showed a more marked difference between the extremes of the valley and the crest. Acting on this suggestion, we examined the earlier charts and found that the same phenomenon could be observed more or less clearly on many of them. Special experiments were made with reference to this point, and when the results were charted it was evident that a vasomotor rhythm of the heart was undoubtedly present in the case of the four subjects examined with regard to this question.

The general nature of the waves and their persistence over a considerable time can be seen in Table II, on page 216. The numbers give in order the lengths of successive heart beats in fiftieths of a second. Each line represents a wave. They are so arranged that the crests of the waves come in the central column. It will be seen that the respiratory rhythm is also always present and in many cases tends to disturb the regularity of the vasomotor wave. In the fourth, fifth, and last lines, for example, the respiratory hollow coincides with the vasomotor crest, and as a result the beats on either side are longer than the one in the centre. Similarly, the last beat of a wave frequently happens to be longer than the one preceding it, because a respiratory crest coincides with the vasomotor hollow.

The facts of the table are shown much better in Fig. 6, which was constructed from the figures obtained from the same experiment. This chart was made in the same way and upon the same scale as the respiration charts given above. The ordinates represent the length of heart beats, one fiftieth of a second to the centimetre, and the abscissas the time of the beginning of the beats in a ratio of one second to the centimetre. A rise of the curve denotes a slowing of the heart, a fall in the curve a quickening.

The subject G. was breathing quietly, and it will be seen that the respiratory effects are clearly marked, but the vasomotor rhythm is on the whole the more prominent. There was probably little mental disturbance, for it was the second day that G. had acted as subject,

TABLE II.
The Vasomotor Heart Rhythm. Experiment 16 on G. Oct. 9, 1899.

Highest.															
		36.5	37.5	38.0	41.0	42.5	45.5	44.0	40.5	40.5	41.0				
		37.0	40.0	42.5	40.5	41.5	42.5	40.0	40.0	41.5	38.5	39.5	39.0	37.5	38.5
			37.0	37.0	38.0	39.0	40.5	38.5	40.0	40.0	39.0				
36.5	36.5	38.0	37.5	37.5	41.5	42.5	40.0	41.0	42.0	38.0	38.0	39.0			
36.5	37.5	39.0	38.0	38.5	41.5	41.5	39.0	39.5	41.0	40.0	38.0	38.0	38.0	36.0	36.0
	35.0	35.0	37.0	38.0	37.5	37.5	42.0	39.0	38.0	38.5	39.5				
36.5	37.5	40.0	41.0	41.5	41.0	41.5	43.0	39.0	38.0	38.5	38.0				
37.0	39.5	39.5	39.0	39.0	39.0	39.0	40.5	37.0	37.5	37.5	35.5	37.5	36.0		
		34.0	34.0	37.5	39.5	41.0	42.5	39.0	40.0	40.0	38.0	36.5	36.5		
		36.0	36.0	38.0	40.0	42.0	39.5	41.0	42.0	39.5	38.0				
36.6	37.2	37.2	37.7	38.7	40.1	40.8	41.5	39.8	39.9	39.4	38.3	37.9	37.8	36.7	37.2

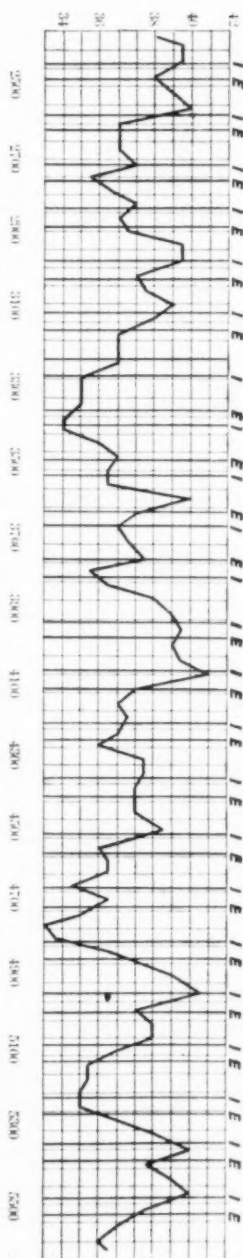
and he had been sitting quietly for some time before the experiment began. The corresponding plethysmogram showed a few long oscillations of volume, but they were not present at all points of the curve.

This chart exhibits seven well marked vasomotor changes in the rate of the heart. The length of the waves is from eight to twelve seconds. The variations in the length of a single pulse at the top and at the bottom of the same wave ranges between eight fiftieths (41-33) and five and one half fiftieths of a second (41-35.5). Or, in other words, the heart in one of these extreme cases had at the crest of the wave a rate of seventy-three per minute and at the base a rate of ninety-one per minute; and in the other, a rate of eighty-five at the crest and of seventy-three at the base.

In general, we find that the variations in the plotted curves of the heart rhythm, both in length and depth, have fairly wide limits. The duration of the heart cycles occurring at the top and at the bottom of a wave may differ by no more than three fiftieths of a second (38-41), but in one exceptional case the longest beat was nearly twice as long as the shortest in the same wave (34-67.5). This last observation exhibited a change in heart rate from eighty-eight to forty-four per minute, although the hollow and the crest in this wave were only three pulse beats apart.

The height of the waves was much greater in warm weather. In the winter months, with a comfortable room tem-

FIGURE 6. Experiment on G., October, 1899. Represents vasomotor rhythm of the heart during quiet respiration.



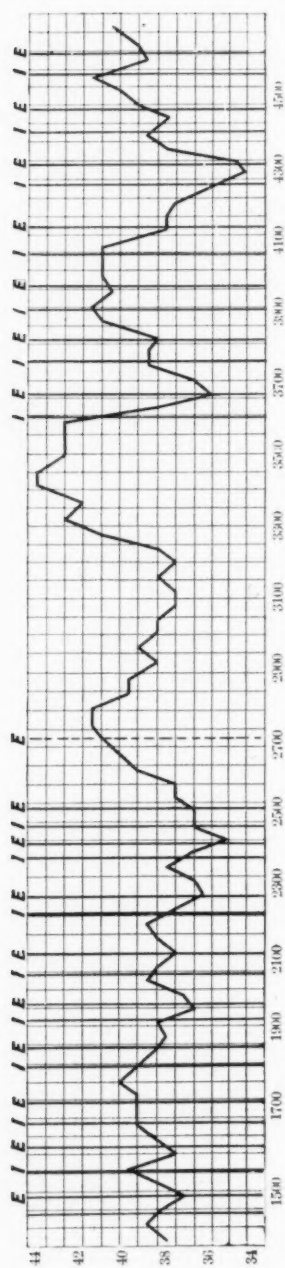


FIGURE 7. On S., October, 1899. An example of increase in the vasomotor waves in heart rate when the breath is held.

perature, the maximum variation was only ten fiftieths of a second (38-48); while in the summer, the variations were quite frequently fifteen fiftieths (30-45) in one subject, and, in the other, variations from twenty to twenty-six fiftieths (34-54, 32-58) were not uncommon, and once, in the instance cited above, the heart rate doubled.

The length of the waves shows a variation in all of our subjects of from about six to fifteen seconds. No regularities in this variation have been made out.

The waves always become much more marked when the breath is held, as can be seen very clearly in Fig. 7, which gives a chart obtained from subject S. This figure is constructed on the same scale as the preceding. It is clear that up to the time that the breath was held there were almost no waves, but that just after there was a quickening of the heart followed almost at once by a sharp slowing and that again succeeded by a quickening. These waves not only lasted throughout the time that respiration was suspended, but continued for a considerable time after the breathing was begun again. This increase in the vasomotor rhythm when the breath is held is interesting in view of the fact that Traube-Hering waves were first noticed on dogs after artificial respiration had been suspended.

Another fact of interest in this connection which has been fre-

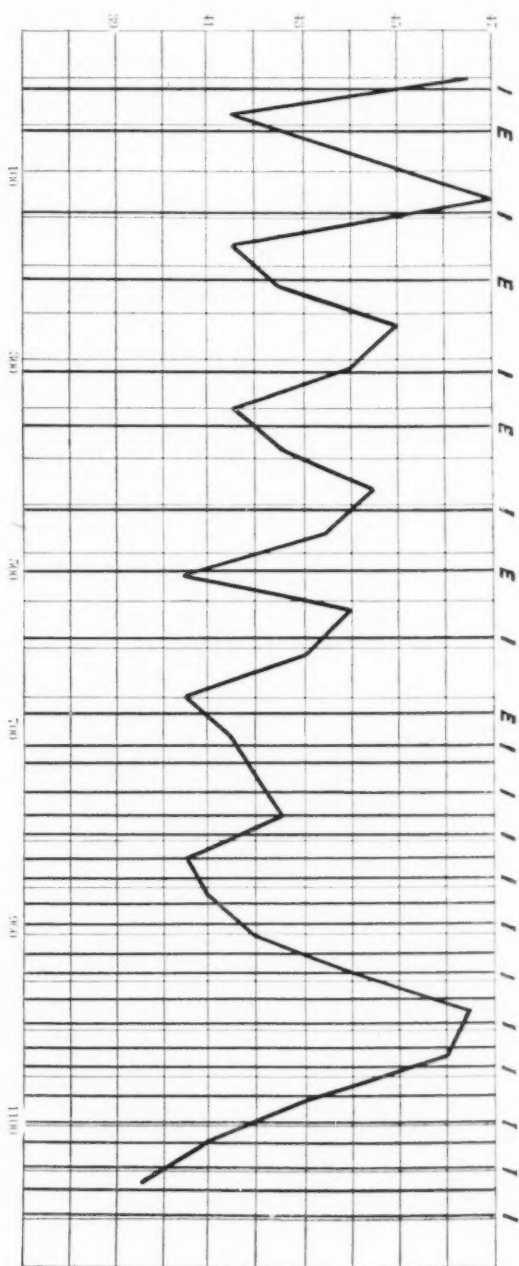


FIGURE 8. On P., August, 1899. An instance of the increase in the vasomotor effect upon heart rate during panting movements.



FIGURE 9 Plethysmogram from middle finger of P.'s left hand.

quently observed on both L. and P. is that the long wave in the heart rate may appear very soon in case of voluntary panting movements. This fact, together with the one noted above, that the waves continue for some time after the normal respiration has been resumed, both after the respiration has been voluntarily stopped and after panting movements, makes it evident that it is not merely dyspnea which conditions the phenomenon. The effect of panting is illustrated, although not in a very striking manner, in Fig. 8.

We have been calling these waves the vasomotor waves of the heart, and it is now time to consider the question of their relation to the supposed vasomotor waves in the volume of the finger. In a plethysmographic tracing it is possible to study the time relation of the two phenomena. Fig. 9 shows a tracing taken from the middle finger of P.'s left hand, and Fig. 10 represents graphically the correspondence of the rhythmic change of length of the succeeding heart cycles and the volume changes in the finger that occurred simultaneously. The upper curve of the chart shows the time and direction of the volume changes in the finger, and the lower curve gives the

heart rate. This chart was constructed like preceding charts except that the time values are in millimetres, as measured from the plethysmographic curve, instead of in fiftieths of a second. On the horizontal axis, ten centimetres equal thirteen seconds, and on the vertical, one centimetre equals one fortieth of a second. It will be noticed that in almost every case the heart begins to quicken just after the volume begins to fall, and continues to quicken until a rise in volume is well under way, then it slows until after the rise of volume reaches its maximum. The vasoconstrictor influence seems to correspond in its action upon the heart to inspiration. Both are ordinarily regarded as more active phenomena than vasodilation or expiration. Another fact of interest in this curve is that the retardation of the heart progresses much more quickly than the acceleration. The former continues through from two to six beats, the latter from seven to twelve. Not all the plethysmograms showed volume changes where the long vasomotor

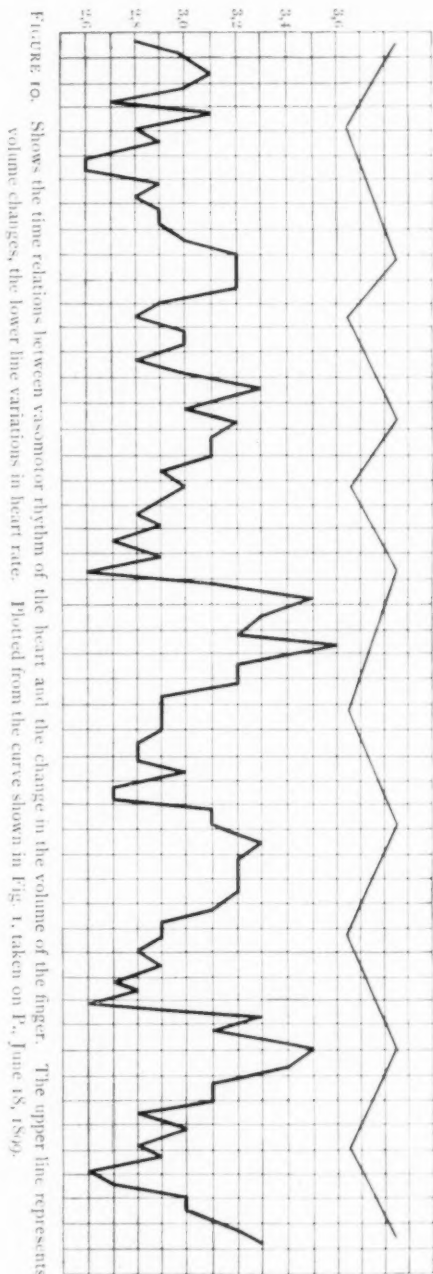


FIGURE 10. Shows the time relations between vasomotor rhythm of the heart and the change in the volume of the finger. The upper line represents volume changes, the lower line variations in heart rate. Plotted from the curve shown in Fig. 1, taken on P., June 18, 1899.

rhythm of the heart was present, but wherever the volume changes could be made out, they corresponded to the heart rhythm and the relation was the same as appears in Fig. 10.

THE ORIGIN OF TRAUBE-HERING WAVES.

Almost all the observations on this subject have been made on animals. The records of arterial blood pressure obtained from animals show three forms of periodic oscillations. Fredericq¹ describes them as, —

1. Oscillations of the first order, the pulse movements.
2. Oscillations of the second order, respiratory effects.
3. Oscillations of the third order, which are longer, and outlast several respiratory oscillations, called by Sigmund Mayer, "spontaneous (or vasomotor) oscillations."

Fredericq objects to Mayer regarding the third class, as "Traube-Hering" waves, for he thinks that it was the second class that Traube and Hering studied. He would give Mayer's name to the oscillations of the third order. It is difficult to decide this question, for under certain circumstances the respiratory waves may be as long as short waves of the third order. Hering² considered that the waves which he studied were caused by rhythmic changes in the tonicity of the blood vessels. He referred the rhythm to the respiratory centre and thought that the periodic innervation of the nerves of the respiratory muscles was accompanied by a simultaneous innervation of the nerves of the heart and blood-vessels. Mayer³ studied not only the blood pressure waves of the second order, but those of the third order. He considered, wrongly, as Fredericq believes, that they were the same that Traube and Hering studied, and he agreed, as he supposed with Hering, when he attributed them to irradiations from the respiratory to the vasomotor centre. He explains the difference in the rate of the respiratory movements and these blood pressure waves on the basis that the tonic action of the vasomotor centre is rhythmically increased by a summation of impulses spreading to it from the respiratory centre. A number of others have written on this subject, —

¹ FREDERICQ: *Archiv für Physiologie*, 1887, p. 351.

² HERING: *Sitzungsberichte der königl. Akademie der Wissenschaften zu Wien*, 1869, Ab. 2, pp. 829-836.

³ MAYER: *Ibid.*, 1876, Ab. 3, pp. 281-306.

Latschenberger and Deahna,¹ Cyon,² and Knoll.³ Knoll's paper seems to have been the result of much careful study, and his conclusions are of interest because they are opposed to the view of Hering and of Mayer, the most generally accepted one to-day. Knoll emphasized the fact that the breathing movements may take place at very different rates, and that the duration and intensity of the long blood pressure waves does not alter to correspond. Lately, Wood⁴ has reported that the rhythmic action of the vasomotor centre is continued after the animal has been put under the influence of veratrum viride, which, he says, destroys the respiratory centre, while it leaves the vasomotor centre unaffected. Lewandowsky,⁵ however, in reviewing his article, says that he misses the proof that the drug has an action on the respiratory centre, rather than on the peripheral nervous respiratory mechanisms; according to Cushney,⁶ however, Wood's view is correct.

Nearly all the writers who have reported on plethysmograph experiments on man have observed variations in the volume of the member studied lasting over several respirations. These long periodic changes in volume have been generally attributed to vasomotor activity, but apparently no definite conclusion has been reached with reference to the origin of the stimuli producing these changes.

The only instance which we have been able to find of any observation, either on animal or man, relating to a correspondence between heart rate and vasomotor activity, is in a foot-note in an article by Binet and Courtier.⁷ In this note the authors state,—"In the case of certain subjects presenting vasomotor oscillations (Mosso), we observe at the moment of the phase of descent of the undulation a slowing of the pulse; this retardation is not of respiratory origin, for the undulation corresponds to three or four respirations. We give a specimen of these undulations in Fig. 25." The statement and the curve are the opposite of the facts thus far observed by us, in that we find a quickening of the heart while the volume of the finger is lessening.

¹ LATSCHENBERGER and DEAHNA: *Archiv f. d. ges. Physiol.*, 1876, xii, p. 200.

² CYON: *Ibid.*, 1874, ix, p. 499.

³ KNOLL: *Sitzungsberichte der königl. Akademie der Wissenschaften zu Wien*, 1885, xcii, Ab. 3, p. 439.

⁴ WOOD: *This journal*, 1890, ii, p. 352.

⁵ LEWANDOWSKY: *Centrallblatt für Physiologie*, 1890, xiii, p. 218.

⁶ CUSHNEY: *Pharmacology and Therapeutics*, 1890, p. 331.

⁷ BINET and COURTIER: *L'année psychologique*, 1895, ii, p. 124.

Most investigators seem to agree that the long "Traube-Hering" waves of blood pressure and the long oscillations in the volume of the limbs, are caused by periodic changes in the tension of the muscles in the walls of the blood-vessels, and that this change of tension is the result of periodic activity of the vasomotor centre. The point of difference is whether the rhythm of the vasomotor centre is of independent origin or a result of irradiations from the respiratory centre.

It was not our intention to study the nature or cause of vasomotor waves of the "Traube-Hering" type, but as our results seem to throw some light on these disputed questions, we have thought it best to refer to it.

It seems to be quite definitely proved that the shorter rhythmic oscillations of the heart rate which accompany respiratory movements are due to irradiations from the respiratory centre upon the cardio-inhibitory centre. Reasoning from analogy, we should be led to expect that the vasomotor centre, like the respiratory centre, might have an independent rhythmic action and might, like the respiratory centre, irradiate impulses upon one or both of the heart centres which would alter the rate of action of the heart.

Our experiments show that the rate of the heart beat does undergo long periodic variations corresponding in general in its time relations with the long changes in the volume of the limbs and the "Traube-Hering" changes in blood pressure, which are regarded as expressions of phases of activity of the vasomotor centre. If, as we believe, the rhythmic changes in the rate of the heart can be taken as an evidence of the activity of the respiratory and vasomotor centres, the view that the long "Traube-Hering" waves are due to irradiation from the respiratory centre, first suggested by Hering, and later supported in modified form by Mayer, is untenable.

If the vasomotor rhythm be due, as Mayer thinks, to summation of stimuli irradiated from the respiratory centre upon the vasomotor centre, there would be a correspondence between the activity of the respiratory and vasomotor rhythms of the heart. An increase in the rate and depth of the respirations would be accompanied by correspondingly increased vasomotor changes, and a decrease in the rate and extent of respirations would be accompanied by lessened vasomotor effects. This correspondence, as Knoll observed for blood pressure changes, and we ascertained for heart rate, does not exist. For example, Fig. 7 shows that while the breath was held, the vaso-

motor effects on the heart were greatly increased, although the respiratory effects were smaller than usual. In other experiments, when the respiratory rhythm was marked, the heart showed almost no recognizable vasomotor rhythm (see Fig. 1). In voluntary panting movements (see Fig. 8) the activity of the respiratory centre, as shown by the extent of the respiratory rhythm of the heart, at first lessened and then failed to appear, while the vasomotor effect became suddenly very prominent. Similar results we have seen many times, and we have in our possession charts which show these special effects more strikingly than those which we publish. As the vasomotor mechanism undoubtedly has a different function from the respiratory, we have no reason to expect that the two would always act at a given time to the same degree.

Our argument is based on the idea that the heart rate can be taken as evidence of the activity of the respiratory and vasomotor centres. This we believe, but we have no absolute proof that the long changes in the rate of the heart are the result of irradiation from the vasomotor centre upon the heart centres, for there is another possible explanation of the change accompanying vasomotor oscillations. It is possible that when the volume of a limb is lessened by vasoconstriction, more blood may be driven into the veins, and the heart receiving a larger blood supply may beat faster. The results recorded in Fig. 10 are in some respects in favor of this view. Thus, one may argue, the heart as a rule does not begin to quicken until vasoconstriction is under way; the heart continues to quicken after vasoconstriction has ceased; and the heart does not begin to slow until vasodilation is well developed. Had both the vasomotor changes and the alterations in the heart rate a common cause, they should, except for slight differences due to reaction times, vary together. The fact that the heart continues to quicken after vasoconstriction has ceased, and does not slow until dilation is well under way, is hard to reconcile with the view that these two phenomena have a common cause. The difference in their time relations can be explained, however, on the assumption that the quickening of the heart is only an indirect effect of the activity of the vaso-constrictor centre, and that it is due to the increased blood supply to the heart which results from it.

This argument is not altogether satisfactory, because the heart sometimes begins to quicken before the volume of the finger has decreased appreciably, and always before the constriction has become

enough for any considerable increase in the blood supply to reach the heart.

These facts make it probable that the heart rate is primarily increased through irradiation from the vasomotor centre upon the vagus or, less probably, the accelerans centre, and that the increase is continued after the vasoconstrictor centre has ceased to act, by the effect of the larger blood supply to the heart resulting from the constriction of the peripheral vessels.

Of course one cannot help asking himself whether an inhibition of the vagus centre, or an excitation of the accelerans centre, caused by irradiation from the vasomotor centre may not outlast the period of irradiation, and whether this may not explain the fact that the heart continues to quicken after the vasoconstrictor influence has ceased. No answer can be given to this question.

It is worthy of note in this connection, that the respiratory effect upon the heart, which the later authors generally agree to be of central origin, may show a similar divergence. Not infrequently the quickening of the heart outlasts the constrictor effect.

A comparison of the time of the respiratory shrinkage of the volume of the limb and the change in heart rate may lead to false impressions if one does not bear in mind that the muscles in the walls of the blood vessels are of the non-striated type and have a long reaction time. In experiments on the effect of sensory stimuli to cause a shrinkage of a limb we have found that about three seconds elapse before the effect of the excitation shows itself. This observation is in accord with the results which have been obtained by François-Franck,¹ Sewall and Sanford,² and Binet.³ The effect of inspiration upon the blood vessels, which, in accordance with the view expressed by the several writers named, we supposed to act through irradiation from the respiratory centre on the vasomotor centre, shows a similar latent period. The constrictor effect resulting from inspiration does not show itself until some time during the following expiration, the delay being about three seconds.

Without wishing to commit ourselves by a definite statement, and admitting that further work is necessary before definite conclusions can be reached, we may say that as a result of our study we have arrived at the following provisional conclusion. The active

¹ FRANÇOIS-FRANCK: *Travaux du laboratoire de Marey*, 1876, ii, p. 1.

² SEWALL and SANFORD: *Journal of physiology*, 1890, xi, p. 179.

³ BINET: *L'année psychologique*, 1896, iii, p. 10.

phases of both the respiratory and vasomotor centres (inspiration and vasoconstriction) are accompanied by irradiation upon the vagus centre of the heart, of a type to inhibit its action and so to quicken the heart. This quickening effect favors the onward movement of blood, which tends to collect in the large veins during expiration, and this effect is enhanced and continued by the increased supply of blood coming to the heart as a result of the contraction of the peripheral vessels.

Even if our view that the long periodic variations in the heart rate are the result of the direct effect of an irradiation from the vasomotor centre upon the heart centre is not correct, and if the increase in the rate of the heart accompanying vasoconstriction is only an indirect effect of the action of the vasomotor centre through an increased blood supply to the heart, our contention as to the cause of the long "Traube-Hering" waves still holds good. In any case the vasomotor change in the heart rhythm must be referred finally to the activity of the vasomotor centre, and the lack of correspondence between respiratory and vasomotor effects is a proof that the long "Traube-Hering" waves are not due to an effect of the respiratory centre upon the vasomotor centre.

SUMMARY OF RESULTS.

A. The Respiratory Effects.

The rate of the heart beat is increased during inspiration and decreased during expiration.

This is a constant phenomenon in normal men.

It occurs during quiet respirations.

The effect is strengthened when the respiration is somewhat prolonged and deepened.

The effect may persist when respiration is voluntarily suspended.

The effect may persist when respiration is suspended by painful reflex excitations.

The time of the respiratory rhythm of the heart may not correspond with rapid (panting) or very slow voluntary movements of the chest.

After such modified respiratory movements of the chest have ceased, the respiratory movements of the chest soon fall into step with the respiratory phases of the heart rhythm.

B. The Vasomotor Effects.

The rate of the heart beat begins to quicken just after the volume of the finger begins to shrink, and continues to quicken until the follow-

ing increase of volume of the finger is well under way, when it begins to slow.

This phenomenon is frequently present in normal men during quiet respirations.

The effect is increased when the breath is voluntarily held, although the respiratory effect on the heart is then lessened.

The effect is increased during voluntary panting movements.

The effect continues for some time after respirations voluntarily modified in these ways have become normal.

The vasomotor rhythm of the heart is most pronounced in warm weather.

CONCLUSIONS.

The secondary rhythms of the heart are due to the overflow of impulses which originate in the respiratory and vasomotor centres; these act upon the vagus centre. Inspiratory activity of one of these centres, and vasoconstrictor activity of the other, act alike to inhibit the inhibitory centre of the heart and thereby quicken the heart rate. The time of action of these two sets of influences as well as their intensity is of importance, for they may aid or oppose each other and produce correspondingly large or small effects.

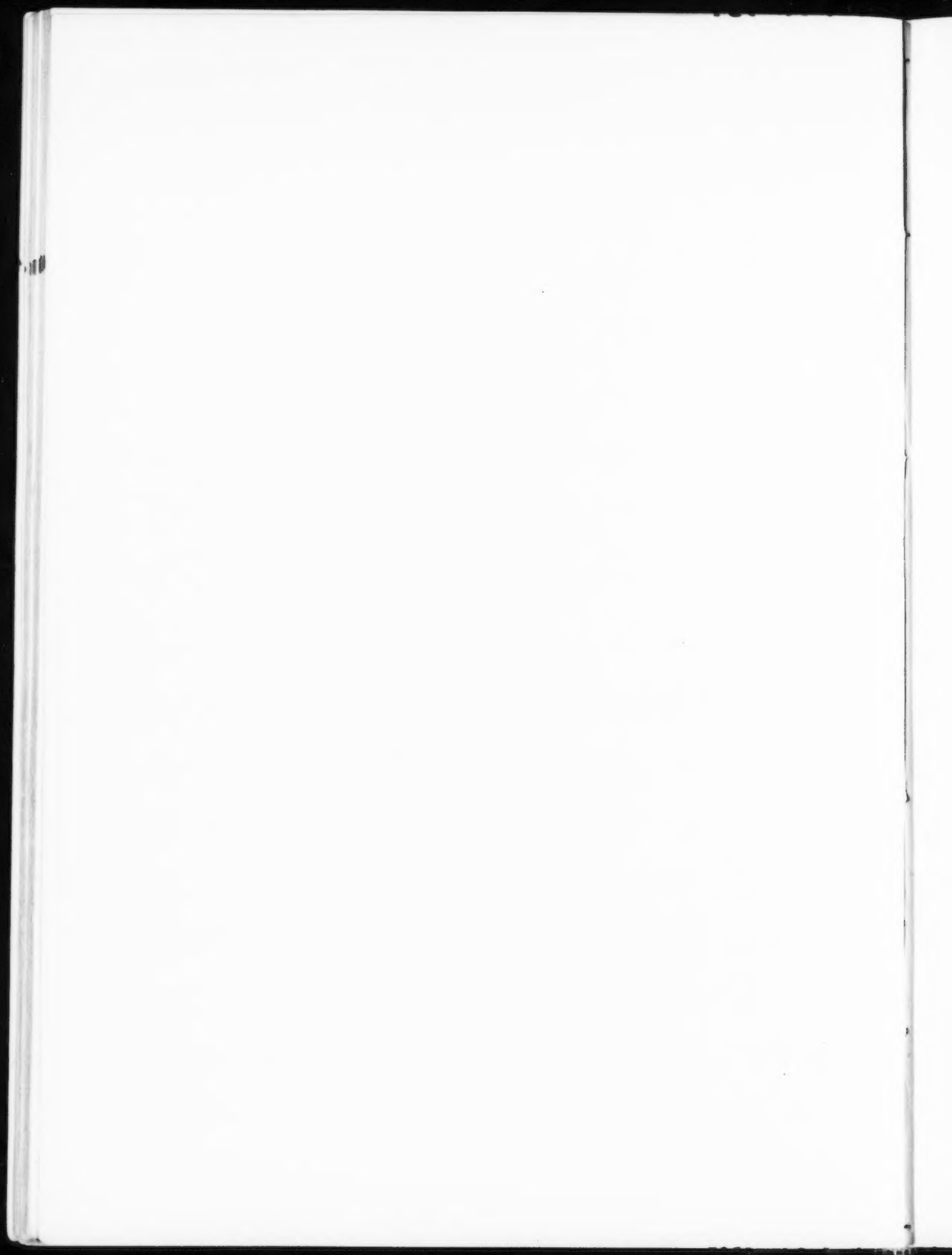
Though corresponding in general in time, the vasomotor changes of the volume of the finger of the Traube-Hering type and the vasomotor rhythm of the heart do not stand primarily in causal relation to each other, but each is the result of the rhythmic activity of the vasomotor centre.

Although our results do not definitely show this, we favor the view that the respiration centre, by irradiation to the vasomotor centre is, in part at least, responsible for the respiration changes in the volume of the finger, etc.

If the respiratory centre can influence heart and vasomotor centres one should expect to find the vasomotor centre acting on the respiratory as well as the heart centre. Possibly the phenomenon known as the Cheyne-Stokes respiration may find this its explanation.

Voluntary modification of the respiratory rhythm acts only in part through the respiratory centre and, if decided, chiefly on the centres in the spinal cord which control the respiratory mechanism.





STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. V.—ON THE MOVEMENTS AND MOTOR REFLEXES OF THE FLAGELLATA AND CILIATA.¹

BY HERBERT S. JENNINGS.

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I. INTRODUCTION.

IN the second of these Studies² I have described the mechanism of the motor reactions to external stimuli in the ciliate infusorian *Paramecium caudatum*. As there set forth, this animal has a fixed form of motor reaction to stimuli of all classes, which, expressed in general terms, is as follows: When unstimulated the animal swims with a certain structurally defined end (the "anterior") in front.

¹ Scientific Results of a Biological Survey of the Great Lakes, directed by Jacob Reighard, under the auspices of the U. S. Fish Commission, No. V. (Published by permission of the Hon. George M. Bowers, Commissioner of Fisheries.)

² JENNINGS: Studies, etc., II. On the Mechanism of the Motor Reactions of *Paramecium*, this journal, 1899, ii, pp. 311-341.

When stimulated motion takes place with another (the "posterior") structurally defined end in front, followed by turning toward one side which is structurally defined and invariable (the aboral in *Paramecium*), — finally succeeded by motion with the same end in front as at first. As thus expressed, the direction of motion after a stimulus has no relation to the nature or the position of the stimulating agent.

This method of reaction has a fundamental bearing on the interpretation of the life phenomena of these creatures, and the question is an important one as to how widespread such a style of reaction is among unicellular organisms. In the third of these Studies,¹ an account is given of the reaction methods of the ciliate infusoria *Spirostomum ambiguum* and *Stentor polymorphus*, in which it is shown that these animals react in essentially the same manner as *Paramecium*. Owing to the larger size of these animals, it was possible to localize the action of stimuli very precisely, so that, especially with *Spirostomum*, even more sharply defined results were obtained than with *Paramecium*. It was found (1) that *Spirostomum* reacts to both chemical and mechanical stimuli, *whether applied to the posterior end, the anterior end, or the side, by swimming backward* (followed by turning); (2) that the turning is always toward the *aboral* side, whether the stimulus occurs at the anterior end, the posterior end, *the oral side, or the aboral side*. All relation of the direction of motion to the localization of the stimulus seems thus excluded.

I have since made a study of the reactions of a considerable number of other Protozoa, representing various groups of the Flagellata and Ciliata, bringing out thus the more widely distributed reaction methods among these animals, as well as discovering for certain Protozoa modifications in important points of the general reaction method described in the two papers above referred to. The results of this study are given in the present paper.

It will be well to define sharply the main questions to which, in the study of a given Protozoön, answers are desired. These are as follows: —

1. Has the given Protozoön a fixed formula for motor reactions, similar to that of *Paramecium*?
2. After stimulation, does the organism always turn toward a certain structurally defined *side*, without regard to the nature or position of the stimulus?
3. After stimulation does the organism always move, before turn-

¹ JENNINGS: *American naturalist*, 1899, xxxiii. pp. 373-389.

ing, toward a certain structurally defined *end* (the "posterior") without regard to the nature or position of the stimulus?

These questions will serve as directives for the observations and experiments, which will not however be in any way limited by them. I give first a detailed account of the reaction methods of the different Protozoa studied, arranging them according to the groups to which the animals belong. This is followed by an analysis and summary of the observations, showing the variations in the motor reactions among the different Protozoa, and giving as far as possible the answers to the questions proposed above, as well as other general conclusions to be deduced from the facts presented.

II. FLAGELLATA.

The majority of the members of this group are very small and without striking differentiations of structure. It is therefore in most cases very difficult to determine whether the direction of motion after stimulation does or does not have any fixed relation to structural features of the animal's body. A study was made of the reactions of a few species, the results of which are presented herewith.

Chilomonas paramecium Ehr. — *Description.* — *Chilomonas paramecium* (Fig. 1) is a minute Flagellate which can always be procured in practically unlimited numbers by allowing aquatic vegetation to decay in water. In cultures of this sort *Chilomonas* is one of the first forms to appear: it is often present in such multitudes as to give the water a milky appearance. The great abundance in which it can be procured at any time make it a most favorable species for experimental studies on the Flagellata. The animal is of an irregularly oblong form, compressed sideways. One end (the "anterior") is usually broader than the other, and bears a shallow notch lying between two "lips." One of these lips (the "dorsal") is much larger and more prominent than the other, so that the anterior end presents the appearance of being obliquely truncate with a notch in the truncate border. From the notch, just beneath the larger lip, arise the two flagella, each of about the same length as the animal's body. The body is sometimes slightly curved, the concavity of the curve being on the so-called dorsal side. All these relations are shown in Fig. 1.



FIGURE 1. *Chilomonas paramecium* Ehr., right side, after Butschli. *a*, anterior end; *p*, posterior end; *d*, dorsal side; *v*, ventral side.

Movements.—If a large number of specimens of *Chilomonas* are mounted and observed under the microscope, they will be seen at first to dart swiftly through the water, — so swiftly that the eye can with difficulty follow them. Some individuals soon come suddenly to rest: others follow, and after a time nearly all the animals are resting against the substratum, beginning movement again only when stimulated. When at rest, *Chilomonas* is attached by one of its flagella, which is thrown in a coil on the surface of the substratum, while the other may or may not retain a vibratory motion.

More exact observation of the movement brings out the following points. *Chilomonas* as it swims forward revolves on its long axis in such a way as to describe a path which is a spiral of some width. By introducing the animals into a gelatine solution, so as to compel them to move very slowly, it can be observed that the position of the body bears a constant relation to the axis of the spiral, the lower or smaller lip being always directed toward the outside of the spiral.

Reactions to stimuli.—(1) If to the quiet *Chilomonas* the faintest possible stimulus be given, as by gently agitating the water, or in any way loosing its hold on the glass, it merely resumes its usual motion, already described. In a certain sense this usual forward motion may then be considered (taking the resting individual as a starting point) as a reaction to the weakest possible stimulus. The motion seems then to be maintained without further stimulus, until the stimulus of contact with a solid (thigmotaxis) again induces the resting condition. The same facts, in accordance with which the usual motion of the animals may be conceived as (at first) a reaction to a weak stimulus, exist in the case of all the organisms studied, and will not be especially mentioned for each. It is usually very difficult to give the resting individual so weak a stimulus as to induce at first only the usual forward motion: as a rule the reaction takes the form to be described immediately.

(2) If now the swimming or resting *Chilomonads* be stimulated beyond this faintest degree, as by jarring the preparation strongly (mechanical stimulus), by letting the swimming animals come in contact with a diffusing chemical, or dropping them directly into a solution of some chemical (chemical stimulus), reaction takes place as follows. The animals dart swiftly *backward*, then *turn sideways toward the smaller lip*, then swim forward in the new path thus determined by the position of the smaller lip. At the moment of turning toward the smaller lip, the flagella, or at least one of them,

are turned toward the dorsal side, over the upper lip, as indicated in Fig. 2.

The point of essential importance here is of course the fact that the animals always turn when stimulated toward the smaller lip, without regard to the nature of the source of stimulus or its position. This is easily seen when the Chilomonads are examined in the thickened gelatine solution, in which all their motions are slow; it is much less easily made out in the animals swimming freely in water, though in all cases observed in which it was possible to determine under these conditions in which direction the animal turned, it was evident that this was toward the lower lip.

The method of reaction is very easily observed under the following conditions. A drop of water containing the animals is spread out in a thin layer on a slide and is left uncovered. The animals in this thin layer of water may then be watched with the high power of the microscope. As the water evaporates, the partial desiccation of the individuals near the edge of the drop acts as a stimulus; they dart backward, turn toward the smaller lip, swim forward, and repeat the operation indefinitely. At this time the layer of water has become so thin that the animals can swim in only one plane and are forced to lie upon one side; it is therefore at once apparent in which direction they turn, — in every case toward the smaller lip.

As in the case of *Paramecium*, the different parts of the reaction may be modified in intensity and duration by different conditions. *Chilomonas* usually swims backward only a short distance, but the distance thus travelled varies somewhat with the intensity of the stimulus, being greater with a powerful stimulus. As the animal swims backward it usually does not revolve at the same time on its long axis, though in the more extensive backward excursions it may do so. The amount through which the animal *turns* is very variable; with a faint stimulus there is a turn of but a few degrees toward the lower lip, while with a powerful stimulus, as when the animals are dropped directly into a strong chemical, or partially desiccated in the manner described, they may whirl about so as to describe a circle several times. When dropped into a weak solution of sodium chloride the entire reaction is repeated many times, — until finally the turning prevails over the other features, and the animals whirl about until they die.

When a *Chilomonas* swimming forward comes in contact with the edge of a drop of some diffusing chemical, as $\frac{1}{5}$ per cent sodium

chloride, the reaction is given as above described, — the *Chilomonas* after reaction of course continuing to swim forward in a new direction. If this new direction, as frequently happens, again brings the animal against the edge of the drop, the reaction is repeated, and this repetition is continued until in accordance with the laws of chance, the animal's movement becomes so directed as not to carry it against the edge of the drop. These relations are identical with those described in the second of these Studies for *Paramecium*.

If a crystal of sodium chloride is placed against the edge of a cover-glass beneath which are large numbers of *Chilomonads*, as the

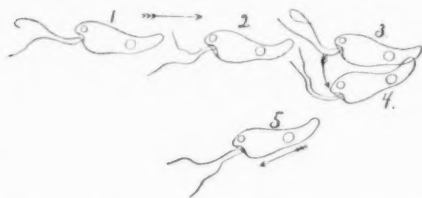


FIGURE 2. Motor reflex of *Chilomonas*. The arrows show the direction of movement, while the numbers show the successive positions taken.

diffusing salt passes under the cover-glass the following effects are observed. The *Chilomonads* affected all swim backward at once. Those whose anterior ends are directed away from the advancing salt solution, swimming backward, pass directly into the solution, and are killed. Those with

anterior ends directed toward the crystal of sodium chloride, likewise swimming backward, move away from the diffusing chemical and therefore escape. Those with the body axis in intermediate directions, swimming backward, move obliquely with reference to the position of the advancing salt solution; some thus escape, while others are killed. The direction of motion bears no relation to the position of the source of stimulus, — the stimulated animals all swimming (at first) simply backward.

The typical motor reflex in this animal may then be expressed as follows: *Chilomonas* when stimulated swims backward, turns toward its lower lip, then swims forward in the direction thus determined. The typical motor reflex of *Chilomonas* is shown in Fig. 2.

A comparison of the facts above given as to the movements of *Chilomonas* with those detailed in the second of these Studies for *Paramecium* reveals a complete agreement in all essential points between the motor reactions of the two, — the one a Flagellate, the other a Ciliate.

***Euglena viridis* Ehr.** — *Description.* — The well-known form *Euglena viridis* (Fig. 3) is an elongated, somewhat fusiform creature, exceed-

ingly flexible, and variable in shape. One end (the "posterior") is pointed; the other (the "anterior") is somewhat obliquely truncate, there being, as in *Chilomonas*, a larger and a smaller lip, with a notch between them. From this notch or mouth projects a single long flagellum. Just behind the larger lip and a little to the left of it lies a red eye-spot.

Movements.—When in undisturbed motion *Euglena* swims forward, revolving on its long axis and describing a slender spiral.

Reactions to stimuli.—When *Euglena* thus swimming is given a weak stimulus, as by its coming in contact with a drop of some weak chemical, or by a mechanical jar to the preparation, it turns toward the larger lip, then keeps on the course so laid out undisturbed. *Euglena* never swims backward under any circumstances, so far as observed. If the

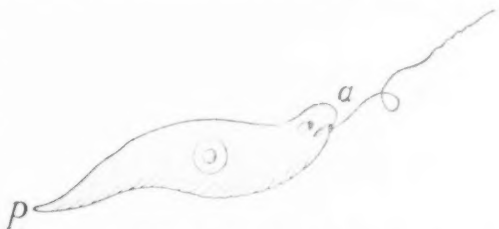


FIGURE 3. *Euglena viridis* Ehr., after Kent. *a*, anterior end; *p*, posterior end.

stimulus is a little stronger, the creature may stop for a moment before turning. If the stimulus is still stronger, the *Euglena* continues turning, so that it frequently revolves thus for a long time, toward the larger lip, without any forward motion at all. If the stimulus continues, the whirling *Euglena* gradually takes a spherical form, the motion ceases, and the creature proceeds to encyst itself. To any very strong stimulus the only response is the immediate assumption of a spherical form and prompt preparation for encystment. This occurs when the creatures are introduced into a chemical solution, as of NaCl, KI, etc., or when allowed to dry on the slide.

The motor reaction of *Euglena* consists then in simply turning toward the larger lip. Further reaction takes the form of preparation for encystment.

In this case then, as in those previously described, the direction of motion after a stimulus is determined by structural differentiations of the body, not by the position of the stimulating agent.

Other Euglenidæ.—Several other species of *Euglena* are common in fresh water; of these *Euglena spirogyra* and *Euglena oxyuris*

were studied. These creatures are much more sluggish than *Euglena viridis*, moving only very slowly and lying quiet on the substratum for long periods; they seem much more vegetable than animal in their nature. In these creatures no definite motor reaction to a stimulus could be observed. When stimulated they either do not react at all, or simply assume the spherical form and make preparations for encystment.

In several species of *Phacus* and certain other green Flagellates no definite motor reaction plan could be made out; these organisms are worthy of further study in this connection.

In these Flagellata we have therefore a gradation in the complexity of the motor reaction to a stimulus. In *Chilomonas* we find all the features exhibited by *Paramecium*, — the swimming backward, turning toward one side, and again swimming forward; continued or severe stimulus causing repetitions of the reaction. In *Euglena viridis* the swimming backward is omitted, the creature merely turning toward one side when stimulated; continued or severe stimulus induces encystment. In *Euglena spirogyra* and *E. oxyuris* even the turning is omitted, there being little or no response to the stimulus but immediate preparation for encystment.

III. CILIATA.

A. Holotricha: *Paramecium caudatum* Ehr. — An extended account of the motor reactions of this animal has been given in the first, second, and fourth¹ of these Studies.² For our present purpose the

¹ JENNINGS: This journal, 1899, ii, pp. 355-379.

² The results given in section 3 of the fourth of my Studies (this Journal, 1899, ii, pp. 363-371) on the Relation of Chemotaxis to Chemical Composition in *Paramecium* require modification, owing to a chemical misinterpretation as to the factors which give solutions their characteristic properties. My discussion was based on the old ideas that the acid properties of solutions are due to the "acid radicals," the alkaline properties to the metal components. But modern chemistry has shown that the characteristic properties of acids are due to the hydrogen ions contained; of alkalis to the hydroxyl ions. This requires changes in my discussion and conclusions, and renders it probable that little or no significance is to be attached to the results as to the attractiveness of certain salts given in Table II, page 366 (*loc. cit.*): since, as was stated in that paper on page 369, they are inconsistent. The only solutions classified as always attractive in my list on page 362 that are known not to contain hydrogen ions when pure are those of sodium and potassium fluoride, and of potassium permanganate. Tests of the samples of these substances used in my experiments have since shown that these, though

following statement of previous results is sufficient: *Paramecium* when stimulated swims backward, turns toward its own aboral side, and swims forward.

It is, perhaps, well to call attention again at this point to the fact that, taking the resting individual as the starting point, the usual straightforward motion may be considered as the simplest form of reaction to a very weak stimulus. Such a stimulus may most easily be given in the case of *Paramecium* by gently removing with a fine glass spicule the bit of material against which the animal is resting, or by a gentle rolling movement separating the *Paramecium* from this bit of material. Thereupon the animal resumes its forward course. This forward motion, however, continues long after the cessation of the external change which induced it, so that it may be proper to speak of the animal swimming forward in the normal manner as "unstimulated."

In addition to details previously given concerning the reactions of *Paramecium*, I wish to add here certain new results, which are of importance for interpreting the phenomena presented by the reactions of this and other unicellular organisms.

Localized stimuli. — (a) *Mechanical.* — With the new Braus-Drüner stereoscopic microscope of Zeiss, it is possible to apply localized stimuli to *Paramecium*, in the manner described in the third of these Studies (*loc. cit.*) for *Spirostomum* and *Stentor*. Under the Braus-Drüner microscope the *Paramecia* in a watch-glass are touched on various parts of the body with the tip of the finest capillary glass rod. By this means it is found that only the anterior end of the procured as strictly chemically pure from a reliable firm, were contaminated with acid: after proper purification they were repellent throughout. All the solutions in which the *Paramecia* gather, therefore, contain hydrogen ions, and it is doubtless to these that the (apparently) attractive qualities are due, instead of to the anions as stated in the paper. On the other hand, my conclusion that the strong repellent power of other solutions is due to the metal components or kations rather than to the OH-ions seems justified, since of the thirty-six substances classified as having strong repellent powers, all contain such kations, while at most less than a dozen contain OH-ions. It is, however, not impossible that OH-ions are repellent also.

In my discussion and conclusions, therefore, wherever an effect was ascribed to the anions or acid radicals, it should be considered due rather to the hydrogen ions. Of the fourteen numbered paragraphs in which I have summed up my conclusions in this paper (*loc. cit.*, pp. 377-379), three — paragraphs 8, 9, and 11 — are to be modified in accordance with these facts. The other conclusions are not modified.

animal is markedly sensitive. When touched at the anterior end the Paramecium quickly gives the typical reaction previously described. But if any other part of the body is touched, the Paramecium does not react at all: it may be pushed out of its course, but does not of itself change its path in the least, in consequence of the touch.

It must be remembered, that owing to the minute size of Paramecium, it is impossible to give it a blow of any force; the creature is simply pushed aside by any object touching it, — just as a bubble of this size cannot be broken by touching it with a rod, in spite of its extreme delicacy. It is quite impossible to cut, bruise, or otherwise injure Paramecium in this way, so that the lack of reaction is doubtless due to the fact that the touch is too light to be perceived, except when it occurs at the sensitive anterior end.

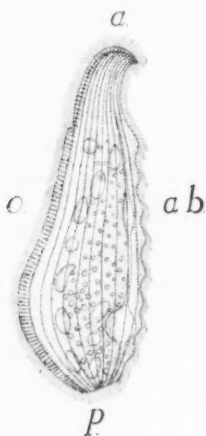


FIGURE 4. *Loxophyllum meleagris* O. F. M., after Butschli. *a*, anterior end; *p*, posterior end; *o*, oral side; *ab*, aboral side.

(*b*) *Localized chemical stimuli*.—For determining the effects of localized chemical stimuli, a capillary glass rod was coated with the chemical to be tested and held near the animals, at one end or one side. The chemical then diffuses, and the effect when it reaches the animal can be noted. It is convenient to use as a stimulus some colored chemical, so that its diffusion can be seen; methyl green serves well for this purpose.

Holding the glass rod thus coated with methyl green *behind* a number of Paramecia, all of which are resting with their anterior-ends placed against a solid (thigmotaxis), it is found that as soon as the diffusing chemical reaches the animals, coming of course first in contact with their posterior ends, they at once *swim backward*, — therefore into the densest part of the solution, where they are killed.

If the methyl green is held in front of them, so that it first comes in contact with the anterior end, the Paramecia likewise swim backward, — therefore *away* from the densest part of the solution, so that they escape.

If a bit of the methyl green or a crystal of sodium chloride is placed in the midst of a group of Paramecia that are oriented in no particular direction, each individual begins to swim backward when the diffusing chemical reaches it. Some thus swim toward the

centre of diffusion of the chemical, others away from it, others obliquely, — exactly as described for *Chilomonas*.

It does not follow, however, from the above experiments that when *Paramecium* swims backward into a chemical solution which first touches its posterior end, this reaction is due to a stimulus at the posterior end. It is possible that when the diffusing chemical approaches the *Paramecium* from the rear, no reaction is caused *till the solution reaches the anterior end*. It is impossible to determine by observation whether this is true or not, even with a colored substance like methyl green, as the *Paramecia* usually begin to react before the

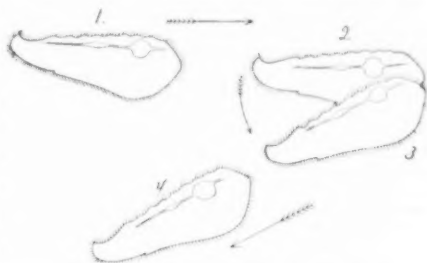


FIGURE 5. Motor reflex of *Loxophyllum meleagris*. The arrows give the direction of motion; the numbers show the successive positions occupied.

color can be seen to have reached them at all. The possibility remains then that in *Paramecia* all motor reactions may be due to a stimulus at the anterior end, — the rest of the body not being sufficiently sensitive to receive a stimulus at all.

***Loxophyllum meleagris* O. F. M. — Description.** — *Loxophyllum meleagris* (Fig. 4) is of a flattened leaf-like form, about three times as long as broad, the anterior end tapering to a point, the posterior end broad. One border (the oral or ventral) is extremely thin; the other (aboral or dorsal) thicker and bearing a series of papillae. The anterior end usually curves toward the aboral side.

Movements. — This animal usually glides along the substratum, lying on one side, — after the manner of the *Hypotricha*. Like the *Hypotricha*, it may also at times leave the bottom and swim freely through the water: at such times it revolves on its long axis from left to right. The gliding motion is the usual one, however.

This gliding motion is either straight ahead or in a gentle curve toward the aboral side, — the latter being evidently due to the curvature of the anterior part of the body. The straight forward movement seems to occur in individuals in which the curving of the anterior end is not marked.

Motor reactions. — When *Loxophyllum* comes in contact with an obstacle of any sort, it turns toward the thin *oral side*. If stimulated

in other ways, as by a mechanical shock, or by a chemical stimulus, it may swim backward a short distance, then turn toward the oral side. The reaction of *Loxophyllum* is represented in Fig. 5.

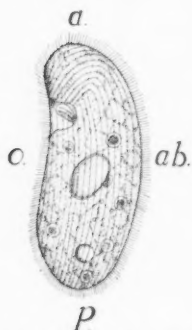


FIGURE 6. *Colpidium colpoda* Ehr., after Schewiakoff, from Bütschli. *a*, anterior end; *P*, posterior end; *o*, oral side; *ab*, aboral side.

It will be noticed that in this case the path of the unstimulated animal takes (frequently) the form of a curve toward the aboral side, while a stimulus causes always a sudden turn toward the opposite, or oral side.

***Colpidium colpoda* Ehr.** — *Description.* — *Colpidium colpoda* (Fig. 6) is somewhat kidney shaped, narrower at the anterior end, two or three times as long as broad, and curved toward the side on which the mouth lies (the oral side).

Movements. — In *Colpidium* we find the same phenomenon in the direction of the normal movement as in *Loxophyllum*, but more pronounced. The animal swims in a strong curve forming the arc of a circle, with the oral side toward the centre of the circle. This is most evident when the animal is following a plane surface, keeping one side against the surface, as it frequently does. When swimming freely through the water it likewise swerves continually toward the oral side, at the same time revolving slowly on its long axis, so that its path takes the form of a wide spiral. The curving of the path toward the oral side seems clearly a consequence of the curved form of the body.



Motor reactions. — When stimulated in any way, *Colpidium* turns toward its aboral side. This may be observed in the usual course of the animal as it glides along a surface. As above stated, its undisturbed path is a curve toward the oral side, but as it at short intervals comes in contact with an obstacle or is otherwise stimulated, it gives a short jerk toward the aboral side. Its path therefore usually takes the form shown in Fig. 7.

FIGURE 7. Path of *Colpidium* when frequently stimulated. The arrows show the direction of motion; the numbers give the successive positions taken.

The reaction is made more evident by using some direct means of stimulating the animals. If a drop of some chemical, as a weak solution of NaCl, is introduced beneath the cover-glass of a preparation of Colpidia, the latter always turn toward the aboral side when they come against the edge of the drop: this is true whether the animals are gliding along a surface or swimming freely through the water. The backward swimming, so noticeable as a part of the reaction in many forms, is nearly omitted here; if the stimulus is strong there is a sudden stoppage before the turning, with at times a slight backward jerk.

If the animals are dropped directly into a solution of a chemical which acts as a stimulus, they begin at once to whirl about, turning continuously toward the aboral side.

Putting all together, the movements and reactions of Colpidium are then as follows: the unstimulated animal swims in a curve which follows the curve of the animal's body, turning therefore continually toward the oral side; at any effective stimulus the animal alters its course by turning toward the aboral side (at times jerking a little backward at the same instant).

Microthorax sulcatus Eng. — *Description.* — Microthorax (Fig. 8) is very small, and somewhat lens shaped, one surface flattened, the other convex and bearing three longitudinal grooves. The anterior end has a blunt point curved toward the ventral edge, the posterior end is rounded. The "dorsal" edge is strongly convex, the ventral edge nearly straight.

Movements. — Microthorax usually creeps or swims on the substratum, lying either on the flat surface or the convex surface; perhaps rather more commonly on the flat surface. The path is not a straight one, but forms a curve, the animal continually turning toward its more convex edge (dorsal). When swimming freely through the water Microthorax revolves continually on its long axis; at the same time it swerves toward the convex edge, so that the path becomes a spiral one.

Motor reactions. — When Microthorax meets an obstruction or comes in contact with water containing some effective chemical in solution, or is otherwise stimulated, it turns with a sudden jerk toward the convex (dorsal) edge, then goes forward again. The sudden turn



FIGURE 8. *Microthorax sulcatus* Eng., after Engelmann. *d*, dorsal edge; *v*, ventral edge; *a*, anterior end; *p*, posterior end.

may or may not be accompanied by a backward jerk. Microthorax never turns toward its straight (ventral) edge.

Dileptus anser O. F. M. — *Description.* — *Dileptus anser* (Fig. 9) is lanceolate in form, pointed at the posterior end, and extending at the anterior end into a long, somewhat trunk-like portion. On one side (the oral) lies the mouth; along the opposite side (the aboral) is a row of contractile vacuoles. The anterior trunk-like portion is slightly curved in such a way that the convexity of the curve lies on the oral side, — the tip being thus directed toward the aboral side.

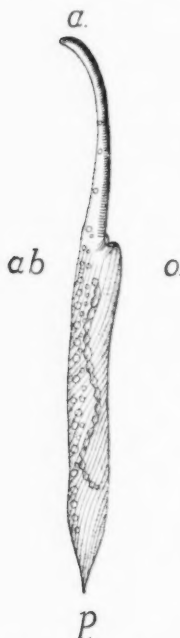


FIGURE 9. *Dileptus anser* O. F. M., after Wrzesniowski, from Butschli. *a*, anterior end; *p*, posterior end; *o*, oral side; *ab*, aboral side.

Movements. — When swimming freely through the water the animal revolves on its long axis in such a way that it describes nearly the surface of a cone, the posterior end forming the apex, while the convex curve of the anterior process (oral side) looks always toward the outside of the cone. In other words, the anterior end swerves continually toward the oral side, the continued revolution carrying the animal forward in a spiral course.

Dileptus may also contract and extend and twist itself about in very irregular ways, though so far as observed the oral side may always be seen to look toward the outside of the irregular curves formed.

Motor reactions. — When *Dileptus* in its forward course strikes against an obstruction or comes in contact with the edge of a drop of some chemical solution, it swims backward, then turns toward the oral side, — toward the convex curve of the proboscis-like anterior region, — then swims forward over the new path so determined.

Rarely the animal becomes so twisted that the convex curve or oral side of the proboscis comes to lie nearly in a line with the aboral side of the remainder of the body. In such a case it is toward the oral side of the proboscis that the animal turns, without regard to the posterior part of the body.

Localized stimuli. — (*a*) *Mechanical stimuli.* — *Dileptus* is of such a size that the effects of localized stimuli may be tried, in the manner above described for *Paramecium*. Touching the anterior end with the spicule of glass, it is found to be exceedingly sensitive, the reac-

tion described above being given at the slightest touch. If the posterior part of the body is touched, usually no reaction is given, showing that, as in *Paramecium*, this part of the body is very little sensitive as compared with the anterior end. By a strong stimulus, however, the animals may be made to react by touching the posterior end, but *they then swim forward*, instead of giving the typical reflex above described. While it seems to be only a small part of the anterior end that is especially sensitive, a sufficiently strong stimulus on any part of the anterior half of the body causes the animal to swim backward, while a strong stimulus applied to any point back of the middle of the body causes *Dileptus* to swim forward.

Thus in *Dileptus* the reaction to a mechanical stimulus varies with the position of the stimulus,—a stimulus in front causing backward motion, a stimulus behind causing forward motion. These results thus differ from those previously obtained for *Spirostomum* (see *Studies*, etc., III, *loc. cit.*); in *Spirostomum* an effective stimulus at any point of the body caused a contraction followed by swimming *backward*.

In *Dileptus* we have therefore a reaction with relation to the localization of the stimulus. But strangely enough this reaction with reference to the localization of the stimulus occurs, as will be shown, (1) only in relation to backward and forward motion, not in relation to movement sideways; (2) it occurs also only in the case of mechanical stimuli, there being no sign of it in the case of chemical stimuli.

(1) As to the first point, we have now to consider the results when the stimulus is applied on one side. If the animal is stimulated on the anterior half of the body, it swims backward, turns to the oral side, and swims forward. If the stimulus is a light one, the backward motion is for only a fraction of the animal's length, when turning takes place. Now, if the animal is stimulated on the oral side, it persistently turns toward that side. The stimulus may be repeated, the animal receiving a series of slight taps; it then turns continually *toward* the rod which is giving the stimuli. If on the other hand a series of stimuli is given in the same way on the aboral side, the animal continually turns *away* from the stimulating agent. There is not the slightest indication of reaction with reference to the localization of the stimulus so far as the direction of turning is concerned. *Dileptus* when stimulated always turns toward the oral side if it turns at all.

(2) As to the second point, we may proceed to an account of the effects of

(b) *Localized chemical stimuli.*—If a crystal of NaCl or a capillary glass rod coated with methyl green is held in front of a resting Dileptus, there is for a moment no response. Then as the diffusing chemical reaches the animal, it darts backward, thus escaping from the advancing flood without injury.

If the chemical is placed some distance behind the Dileptus, as the diffusing chemical reaches it the animal *darts backward*, as before, thus approaching and entering the dense solution, where it may be killed. If the chemical solution comes from one side, the same reaction is given,—the direction of motion being then neither toward nor away from that from which the solution is coming. If the animals are dropped directly into a weak chemical solution, the reaction is likewise the same.

There is thus no sign in the reaction to a chemical of any relation to the localization of the stimulus. It seems probable that no reaction occurs until the diffusing chemical has reached the sensitive anterior end of the animal; then the typical reflex is given without relation to the direction from which the chemical came.

Loxodes rostrum O. F. M.—*Description.*—*Loxodes rostrum* (Fig. 10) is a very large infusorian, of a flattened elongated form, the anterior end pointed and curved toward the oral side; the posterior end likewise pointed or sometimes blunt. One of the sides is convex, the other deeply furrowed. The mouth lies on one edge (the oral), not far from the anterior end. Owing to

its large size and the slowness of its motion, *Loxodes* is an especially favorable form for observing the effects of localized stimuli.

Movements.—*Loxodes* moves very slowly, at times creeping along the substratum on one side, at times swimming freely through the water. In the former case the path is nearly or quite straight; in the latter case the animal revolves and the path is a spiral, the aboral edge lying to the outside of the spiral.

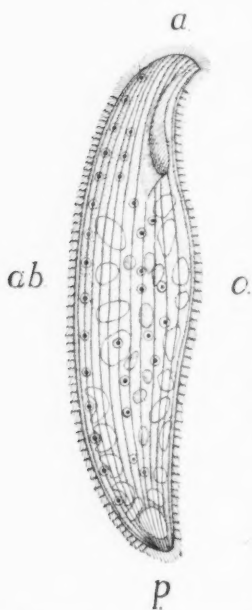


FIGURE 10. *Loxodes rostrum* O. F. M., after Bütschli. *a*, anterior end; *p*, posterior end; *o*, oral side; *ab*, aboral side.

Motor reactions. — When *Loxodes* strikes with its anterior end against an obstruction, or thrusts it suddenly into a solution of some chemical, it swims slowly backward and turns to the aboral side.

Localized stimuli. — (a) *Chemical.* — If a crystal of sodium chloride is dropped near the anterior end of *Loxodes*, it swims backward (thus away), turns to the aboral side, and swims forward. If the salt is dropped near the posterior end, the *Loxodes*, when the diffusing solution reaches it, swims backward, as before. It thus approaches and enters the densest part of the solution, where it may be plasmolyzed and killed.

(b) *Mechanical stimuli.* — Touched on the anterior part of the body with the glass rod, *Loxodes* gives the complete typical reaction. Touched on the posterior part, it swims forward. Touched on the aboral side, the animal turns persistently toward the stimulating agent; on the oral side, it turns away from the stimulating agent. Thus it always turns toward the aboral side.

The facts as to the reactions of *Loxodes* are thus precisely parallel to those of *Dileptus*. The animal reacts with reference to the localization of mechanical stimuli so far as backward and forward motion is concerned, but not so far as movement sideways is concerned. In the case of chemical stimuli there is no relation to the localization of the stimulus.

Prorodon. — In all of the Protozoa thus far discussed, there is a more or less striking asymmetry in the form of the body, and this asymmetry is directly correlated with the method of reaction, so that the direction in which the animal turns after receiving a stimulus may be expressed in terms of the animal's structure. But there are a number of infusoria in which no such asymmetry is to be observed, the animals presenting apparently a complete radial symmetry. Such is the case for example with *Trachelomonas* among the Flagellata: such also is nearly or quite the case with *Coleps hirtus* and with the various species of *Prorodon* (Fig. 11) among the Ciliata. The reactions of a number of such forms were studied with care, but in most cases it was impossible to tell whether the animal does or does not turn after stimulation always toward the same side, — the different sides not being distinguishable.

Finally a large species of *Prorodon* was secured which presented the elements necessary for a solution of the problem.



FIGURE 11. *Prorodon niveus* Ehr., modified from Ehrenberg.

Description.—Prorodon (outlines of a certain species of which are shown in Fig. 11) is oval in form, with the pharyngeal tube at the anterior end, so that it is not possible to structurally distinguish different sides. It is therefore of course impossible to tell, from the structure alone, whether the animal after stimulation always turns toward a definite side or not. But here the food vacuoles come to the rescue. The animals contained numerous food vacuoles of different size and color, and these occupied definite positions within the body. The movement of the food vacuoles was so slow as not to be apparent except upon long observation. By fixing upon one of these vacuoles, occupying a definite position near one side of the animal's body, it was possible to determine in every case the direction of movement of the animal with reference to the position of this vacuole. Upon doing this, it is found that Prorodon, like the other infusoria, always turns toward the same side.

Movements.—The motion of the unstimulated Prorodon is straight forward, at the same time revolving on the long axis to the left.

Motor reactions.—When stimulated it first swims backward, — sometimes a very little, sometimes a long distance. Then it turns. One specimen whose movements were studied contained near one side a large brown food vacuole, — the other food vacuoles being much smaller and less deeply colored. In every case this animal turned after stimulation toward a point in its body surface lying about fifty degrees to the right of the position of this large brown vacuole. In other words, if a plane is passed through this vacuole and the longitudinal axis of the Prorodon, and another plane cutting the first at an angle of about fifty degrees is likewise passed through the long axis, the animal always turned after stimulation in this second plane. Moreover, it always turns in the same direction in this plane, — in a direction definable as follows: when the brown food vacuole lies in the *upper* surface the direction of turning is somewhere in the right half of the animal; if the vacuole is below, the turning is to the left. Similar results were obtained by a study of other individuals.

It thus appears that the radially symmetrical Prorodon likewise always turns toward a definite side. Though anatomically symmetrical, it is physiologically unsymmetrical.

B. Heterotricha: Stentor polymorphus Müller.—An account of the reactions of this animal was given in the third of these Studies (*loc. cit.*). The reaction to a stimulus is essentially as follows: the animal contracts, swims backward, turns to the right, and swims forward.

Spirostomum ambiguum Ehr.—The reactions of *Spirostomum* were likewise described in the third of these Studies. When stimulated, the animal contracts, swims backward, turns toward the aboral side, then swims forward.

In the case of localized stimuli, the *Spirostoma* previously described (*loc. cit.*) reacted in the same manner whatever the part of the body stimulated. Stimuli at the anterior end, at the posterior end, or on one side, caused the characteristic reflex, including the swimming backward. In view of the different results obtained for *Dileptus anser*, *Loxodes rostrum*, and the *Hypotricha*, as detailed in this paper, I have been at some pains to re-examine the reactions of *Spirostomum*. I can confirm the results previously given, from experiments on specimens from several cultures. As noted in the previous account, the posterior end is slightly less sensitive than the anterior end, and the percentage of cases giving the typical reaction was slightly less in the case of stimuli at the posterior end, as compared with the results of stimulation at the anterior end. In specimens from some cultures the difference in reaction to stimuli at the two ends was greater, but in the majority of cases a mechanical stimulus at the posterior end caused the animals to contract and swim backward, exactly as does the same stimulus at the anterior end or the side.

To what is this difference between *Spirostomum* on the one hand and *Dileptus*, *Loxodes*, and the *Hypotricha* on the other, in regard to reactions to localized mechanical stimuli due? *Spirostomum* is a very long slender form, and the posterior part seems relatively much more sensitive than in the other cases; it seems probable that to this greater sensitiveness of the posterior end is due the production of the typical reflex when the posterior part is stimulated. *Spirostomum* differs from most of the others in that it always contracts strongly before responding with the motor reaction; possibly this contraction and the typical motor reflex with its backward swimming are closely bound up together, so that whatever causes the former must cause the latter also.

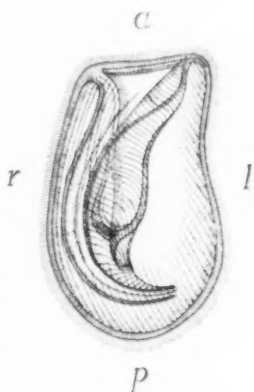


FIGURE 12. *Bursaria truncatella* O. F. M., after Schuberg, from Butschli, ventral view. *a*, anterior end; *p*, posterior end; *r*, right side; *l*, left side.

Bursaria truncatella O. F. M. — This very large Ciliate has proved much less favorable for experimental work than was expected, owing to its extreme delicacy. To the slightest unfavorable influences or mechanical injuries it succumbs quickly, passing first into a pathological condition, then rapidly going to pieces.

Description. — Bursaria (Fig. 12) is ovate, truncate anteriorly, broader and rounded posteriorly. The oral side is flattened, with a very deep groove passing far into the substance of the body; the aboral side is strongly convex.

Movements. — The animal usually swims forward, revolving to the left. At times it jerks back a little (probably as a response to a

slight stimulus); at the same time the revolution may partly or completely stop. At times it swims forward for some time without revolving; at such times the path is in some individuals a straight line, in others a regular curve. The direction of the curve probably depends (as in *Colpidium* and *Loxophyllum*) on the form of the animal's body. In almost all cases observed the path was a gentle curve to the right (the oral side being considered as ventral). In one or two cases of individuals which had been kept for a long time and were possibly

in a pathological condition, the forward path was a curve to the left.

Motor reactions. — Bursaria is excessively sensitive to external influences: at any stimulus it swims backward more or less, then turns to the right, and swims forward. The turning to the *right* as a response to a stimulus is invariable whether the regular course is a straight line, a curve to the right, or a curve to the left. Bursaria confined under a supported cover-glass can detect the difference in the water as it nears the edge of the cover-glass nearly a millimetre from the edge. It then turns, always to the right, — even though its right side already lies next to the edge of the water, so that it is compelled to turn in this direction more than 180° to avoid the edge, while to the left it would have had to turn through but a small angle.

The reaction of Bursaria is represented in Fig. 13.

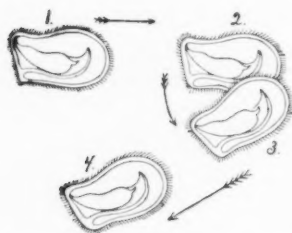


FIGURE 13. Motor reaction of *Bursaria truncatella*. The arrows show the direction of motion throughout the reaction, while the numbers indicate the successive positions occupied.

C. Hypotricha: *Oxytricha fallax* Stein. — *Description.* — Like most of the Hypotricha, *Oxytricha* (Fig. 14) has a strongly dorso-ventrally flattened body, the dorsal surface being convex, the ventral surface plane. The cilia and cirri serving for locomotion are confined to the ventral surface. The mouth is situated on the ventral surface a very little in front of the centre, from it a row of large cilia, the adoral zone, passes forward and to the left as far as the left corner of the anterior margin; thence it passes along this margin to the right corner. The posterior portion of the animal is distinctly broader than the anterior portion. Anterior and posterior ends, right and left sides, and dorsal and ventral surfaces are thus strongly marked and easily distinguishable.

Movements. — *Oxytricha* usually runs along the substratum with ventral side applied to it, by means of the ventral cirri. When running along a plane surface, the path followed is in some individuals a straight line, in others a curve to the right, in others a curve to the left. Whether the path is a straight line or a curve, and in the latter case the direction of curvature probably depends partly or entirely upon the form of the given individual; some specimens in which the body was curved distinctly to the left followed a path that was likewise curved to the left. For a given individual traversing a plane surface the direction of the curvature was constant, so far as observed, although the degree of curvature of the path varies in one and the same animal.

Individuals running about on an irregular surface amid detritus, algae, etc., may follow the substratum in any direction it takes them, running around the surface of spherical or irregular masses, or following a filamentous alga as it curls to the right or left, or up and down. The direction of movement seems determined by the form of the substratum on which the animals are moving.

Besides this usual motion along the substratum, *Oxytricha* may swim freely through the water, at the same time revolving on its long axis.

Motor reactions. — (a) *Mechanical stimuli.* — As *Oxytricha* runs along the substratum it frequently comes in contact with small ob-



FIGURE 14. *Oxytricha fallax* Stein, after Kent, ventral view. *a*, anterior end; *p*, posterior end; *r*, right side; *l*, left side.

structions; it thereupon jerks slightly backward and *turns to the right* (that is, toward its own right side), then again pursues a forward course. Similarly if some moving infusorian strikes in its course against the *Oxytricha*, the latter jerks back and turns to the right. If the preparation containing the moving animals is jarred, they all jerk backward, turn to the right, and move forward. As *Oxytricha* moves along the substratum it will frequently be seen thus to jerk back and turn a little to the right, there being probably invisible

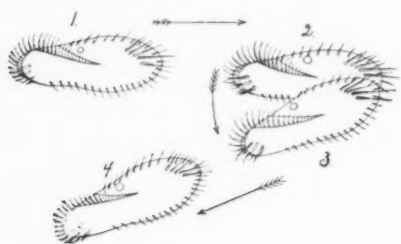


FIGURE 15. Motor reflex of *Oxytricha fallax*, viewing the animal from the ventral side. The arrows give the direction of motion; the numbers show the successive positions occupied.

sources of stimuli affecting the animal. The turning to the right after such a jerk backward is invariable, whether the original path was a straight line, a curve to the right, or a curve to the left. In long continued observation of many individuals I have never yet seen one turn otherwise than to the right after a stimulus. The typical motor reflex of *Oxytricha* is shown in Fig. 15.

(b) *Chemical stimuli*. — If a weak solution of some chemical, as sodium chloride, is allowed to run beneath the edge of the cover-glass, so that there is a sharply defined boundary between the edge of the drop and the water containing the infusoria, many of the latter will in their course come against the edge of the drop. They thereupon turn to the right. This turning to the right occurs both when the animals strike with the anterior end squarely against the edge of the drop, and when they come against it obliquely. Cases were observed in which the *Oxytricha* simply grazed the edge of the drop with its right side; it nevertheless after jerking backward turned to the right. In such a case, if the forward course again leads the animal against the edge, it turns to the right again. It is evident that continued turning to the right will soon bring the animal completely around, so that it will be headed in the opposite direction.

Animals swimming freely through the water and at the same time revolving on the long axis, likewise turn to the right when they come in contact with the edge of a drop of some chemical in solution. If they are dropped directly into a solution of sodium chloride

they jerk backward, turn to the right, and so continue for a long time, — the whole effect being that of a jerky whirling toward the right. Dropped into 6 per cent solution of sodium hypochlorite, they all whirled steadily and smoothly to the right. Some other chemicals cause the animals to swim backward a considerable distance before turning to the right; doubtless variations in the effects of chemicals similar to those described in the fourth of these Studies (*loc. cit.*) for *Paramecium* might be worked out for *Oxytricha*.

Localized stimuli. — (a) *Mechanical.* — Touching various points on the surface of the body with the capillary glass rod, the anterior end is found to be much more sensitive than the rest of the body. The slightest touch at the anterior end — apparently even a touch to one of the cirri of this region — causes a strong reaction. The posterior part of the body is much less sensitive; the tip of the glass rod may even be thrust so violently against it as to leave a visible mark, without any strong reaction being caused. The animal reacts indeed, but quietly as compared with the violent movement when the anterior end is touched.

Touched at the sensitive anterior end *Oxytricha* reacts by darting backward, turning to the right, and swimming forward again (typical motor reflex).

Touched on the posterior half of the body, usually no reaction is caused. If a strong blow is given with the tip of the capillary glass rod sufficient to make a visible mark, the animal responds by *running forward*.

Touched on the left side, the animal jerks back a little, then turns to the right (away from the stimulating agent).

Touched on the right side, *Oxytricha* jerks back a little and turns to the right, as before, — therefore toward the stimulating agent. The rod may give repeated quick taps on the right side; the animal turns persistently to the right, as if following the rod. If touched repeatedly on the left side, it of course turns as persistently away from the rod.

Thus *Oxytricha*, like *Dileptus* and *Loxodes*, reacts with reference to the localization of the stimulus so far as backward and forward motion is concerned, but not so far as motion sideways is concerned: it turns toward a structurally defined side, without regard to the place of stimulation.

(b) *Localized chemical stimuli.* — As already shown, *Oxytricha* responds to a chemical stimulus which first affects the anterior end

or the side by backing a little, turning to the right, then swimming forward, — without regard to which side it is that first comes in contact with the chemical. There remains to be described the reaction to a chemical stimulus coming from behind. If a crystal of sodium chloride or a rod coated with methyl green is placed behind the resting *Oxytricha*, there is for a moment no response; then as the diffusing chemical reaches the animal, the latter at once swims backward. It thus passes into the densest part of the solution and may be killed. Usually after swimming some distance backward, this motion is exchanged for a whirling motion to the right. If the chemical is placed in front of *Oxytricha*, it darts backward, as before, — thus away from the diffusing chemical. If the animals are introduced directly into the chemical, they fall at once to swimming backward, which usually gives place soon to a whirling to the right.

The direction of motion after a chemical stimulus has thus in *Oxytricha* no evident relation to the localization of the stimulus.

Hypotricha in general. — Without determining the species, the reactions of a considerable number of other *Hypotricha* (using this group as limited by Bütschli) were examined. All agree in the main features of structure with *Oxytricha*, and the method of reaction was practically identical in all. At any stimulus, except a mechanical stimulus confined to the posterior half of the body, all the species examined jerk backward more or less, turn to the right, then go forward again. No exception to this turning to the right after a stimulus was seen in any case.

As shown by the foregoing description of *Oxytricha* and other *Hypotricha*, the infusoria of this group present in their reactions certain features which give much complication to their locomotion as a whole. (1) In following an irregular substratum, such as a crooked alga filament, the animals may turn to the right or left or up or down, — the direction being determined solely by the form of the surface on which they are moving. This may be a mechanical result of the method of locomotion. As is well known these animals use a number of large cilia or cirri on the ventral side like legs for creeping. In following an alga bent to the left, for example, on coming to the bend the cirri on the right side would on being pushed forward find nothing to rest upon; the left cirri on the contrary would find a point of support, and thus pulling alone on the body, would necessarily turn it to the left. (2) In the same species some of the unstimulated individuals follow a straight path; others a path which curves to the

left; others a path which curves to the right. Since, so far as observed, the path of a given individual is always curved in the same direction, it seems probable that the curve of the path is due to the form of the body; but the fact that the degree of curvature in the path of a single individual varies indicates the possibility of the presence of other factors in determining the direction of movement.

In spite of this greater complication in the movements of the *Hypotricha* as compared with other infusoria, it is in this group that the reaction to a stimulus by turning in a definite direction (to the right) without regard to the position of the source of stimulation, is peculiarly striking and easily observed. This is of course owing to the fact that the *Hypotricha* do not revolve as they move through the water, as is done by most other infusoria, but run along the bottom with the ventral side below. There is thus no difficulty in determining the direction in which the animals turn after stimulation, and this is invariably toward the right side.

Other Infusoria. — The foregoing discussion contains an account of the reactions of representatives of the chief groups of the infusoria, with the exception of the *Peritricha*. The members of this group are nearly all fixed forms, and hence not fitted for determination of the questions raised in this paper. It is true that such forms as *Vorticella* frequently break from their stalks and swim freely through the water. But the movement seems less well coordinated than in the other infusoria, and to almost every stimulus these creatures respond by sharply contracting the body and folding the adoral cilia inward, at the same time of course ceasing to move. I have not as yet therefore succeeded in determining with certainty any such regularity in their motor reactions as was observed in other infusoria. In addition to the species whose reactions are above described, I have studied many other species which did not, for one reason or another, give clear results, and which were therefore not included in my account. It is of course possible that a complete analysis of the reactions of some of these forms may give results differing in principle from those here detailed; in other words it is possible that essentially different laws of reaction may obtain as between different infusoria. But from the considerable number of representatives of different groups whose reactions were worked out and above described, it is believed more probable that the general principles of the motor reaction methods for the entire group have been obtained. No attempt has been made in the present paper to give an exhaustive account of the reactions of

any of the organisms studied; rather were their reactions examined with a view to the answering of certain definite questions, stated in the introduction.

We will now proceed to an analysis of the observations above recorded and a discussion of the conclusions to be drawn in regard to the nature of the motor reactions of these creatures.

IV. ANALYSIS OF THE OBSERVATIONS, SUMMARY, AND CONCLUSIONS.

(1) The first of the questions proposed in the introduction was, Has the given Protozoön a fixed formula for motor reactions, similar to that of *Paramecium*?

This question has now been answered for representatives of the main groups of Flagellata and Ciliata, — including a Flagellate with two flagella (*Chilomonas*), others with a single flagellum (*Euglenidæ*), representatives among the Ciliata of the Holotricha (*Paramecium*, *Loxophyllum*, *Colpidium*, *Microthorax*, *Dileptus*, *Loxodes*, and *Prorodon*), of the Heterotricha (*Stentor*, *Spirostomum*, and *Bursaria*), and of the Hypotricha (*Oxytricha* and others). In all these species a fixed and definite type of motor reflex was shown to occur, though with much variation in details. In general, when one of these organisms is affected by a decided stimulus, the motor reflex takes the form of a swimming toward one end (structurally defined), turning toward one side (structurally defined), then swimming again with the same end in front as before stimulation. This question is then to be answered in the affirmative, — under the limitations given in the following paragraphs.

In the details of the reflex there is much variation. This may be brought out by noting the difference in behavior when each organism comes, for example, against the edge of a drop of a weak solution of some chemical, as sodium chloride. The lowest motor reaction is shown perhaps by *Euglena viridis*, which simply turns toward a definite side (the larger lip): if stimulation continues, it takes the spherical form and prepares to encyst. There is here no swimming backward and no contraction. A still lower condition is found in *Euglena spirogyra* and *E. oxyuris*, in which there is no motor reaction at all, the organisms simply remaining quiet and beginning the process of encystment at any evident stimulus. Most of the organisms studied gave the typical motor reflex above characterized. In others

(Spirostomum and Stentor, a strong contraction of the body is an additional feature of the reaction.

(2) The second question proposed was, After stimulation does the organism always turn toward a certain structurally defined *side*, without regard to the nature or position of the stimulus?

This question is answered for all the organisms studied in the affirmative. In any given organism the side toward which the creature turns after a stimulus is invariable, and has no relation to the localization of the stimulus.

The side toward which the animals turn is variously defined. In very closely related forms, as in the different *Hypotricha* (Butschli), common structural features define the direction toward which the animals turn (in this case, toward the right side). In more distantly related species it is not possible to name any single structural peculiarity by which the direction of turning can be defined. Thus *Chilomonas* turns toward its lower or smaller lip, *Euglena* toward its upper or larger lip. *Paramecium*, *Colpidium*, *Loxodes*, and *Spirostomum* turn toward the aboral side; *Loxophyllum* and *Dileptus* toward the oral side. *Microthorax* turns toward its "dorsal" edge; *Stentor*, *Bursaria*, and the *Hypotricha* toward the right side. *Prorodon* turns toward one side which is invariable, but not structurally marked. There is of course such great variation in the structure and form of these animals that the same term (as "right" or "aboral") may have very different significations with respect to the general form in different species, so that there is no especial reason for expecting uniformity in this matter. The direction of turning in a given case depends on the structural and functional peculiarities of the locomotor organs, taken in connection with the geometrical form of the body on which they act.

The relation of the direction in which the animals turn after stimulation to the usual direction of locomotion is likewise variable. Most of these organisms, as they swim forward unstimulated, swerve toward one side or the other, — so that a spiral course results, if they at the same time revolve on the long axis. This side toward which the creature swerves may or may not be the same as the side toward which it turns after stimulation. In *Chilomonas*, *Paramecium*, *Microthorax*, *Dileptus*, *Stentor*, *Spirostomum*, and *Bursaria* they are the same; in *Loxophyllum* and *Colpidium* the animals swerve in ordinary locomotion toward one side (owing to the form of their bodies), while after stimulation they turn toward the opposite side. The

Hypotricha may swerve from a straight line either to the right or to the left, but after stimulation the turn is always to the right.

(3) Before taking up the third question proposed in the introduction, it will be well to state briefly the facts brought out in regard to the comparative sensitiveness of different parts of the body. As shown by localizing mechanical stimuli, *the anterior end is much the most sensitive part of the body*. This was demonstrated for Paramecium, Dileptus, Loxodes, Stentor, Oxytricha, and in a less marked degree for Spirostomum; it is probably true for the others also.

(4) The third question proposed in the introduction was, After stimulation does the organism always move, before turning, toward a certain structurally defined *end* (the "posterior") without regard to the nature or position of the stimulus?

This question is answered, in the general form above stated, in the *negative*. Yet the conditions and limitations of this negative are such that an analysis of the observations is necessary for an appreciation of its signification.

(a) As pointed out in the accounts of Chilomonas and Paramecium, the usual *forward* motion of all the organisms above described may be considered a reaction to a stimulus, if the resting condition be taken as a starting point — inasmuch as the beginning of this motion is due to an external change. The resting individual may be induced to resume the forward motion by any very gentle movements such as will separate it from the object against which it is resting, without giving it a stimulus of a pronounced character. It then requires another stimulus to induce again the resting condition.

(b) Some forms, as Euglena viridis, do not swim backward at all even when strongly stimulated, — the typical motor reflex consisting merely of the turning toward a definite side.

(c) For other organisms, the following facts are brought out by the experiments with localized stimuli: —

In Dileptus, Loxodes and Oxytricha, the end toward which the animal moves after a mechanical stimulus depends upon the localization of the stimulus; to such a stimulus at the anterior end the animals react by swimming backward, while if the stimulus is at the posterior end they swim forward.

For chemical stimuli, on the other hand, in the same infusoria, as well as in others, the *absence* of any such dependence of the direction of motion on the position of the stimulating agent was demonstrated.

Since the experiments with localized mechanical stimuli clearly

demonstrate that the organisms just named have the power to distinguish stimuli upon the posterior part of the body from those on the anterior part, and to vary their reaction accordingly, the question arises as to why this power is not exercised in the case of chemical stimuli. The fact that the organisms swim backward when stimulated by a chemical substance diffusing from the rear has cost the lives of many infusoria in these experiments. This perverse and useless method of reaction has been demonstrated to occur in *Chilomonas*, *Paramecium*, *Loxodes*, *Dileptus*, *Spirostomum*, *Oxytricha*, and other *Hypotricha*.

The explanation of this lack of appropriate reaction in the case of chemical stimuli is probably as follows: As stated above, it is proven in many cases that the anterior end is much more sensitive than the remainder of the body, — the latter being comparatively impercipient. Thus a light touch with the glass rod, that would at the anterior end induce a strong reaction in *Paramecium*, *Loxodes*, *Dileptus*, *Stentor*, or *Oxytricha*, produces in these same organisms when applied to the posterior part of the body no reaction at all. Now, suppose a chemical substance diffusing somewhere in the rear of one of these infusoria. As the very dilute solution first reaches the posterior end, it is too weak to act as a stimulus upon this comparatively unsensitive part. The animal therefore remains quiet, and the chemical continues to diffuse, until, coming from the rear, it reaches the sensitive anterior end. Thereupon a strong reaction is induced, which, resulting from a stimulus applied at the anterior end, takes the form of swimming backward, etc., rather than forward. The animal may thus enter the destructive solution and be killed by it.

Spirostomum ambiguum presents an exception to the general rule, in so far that even mechanical stimuli applied to the posterior end usually induce the swimming backward, exactly as when applied to the anterior end. The greater relative sensitiveness of the posterior end of *Spirostomum* and its habit of contracting strongly at any stimulus seem to be connected with this fact. (See the account of *Spirostomum* above.)

To unlocalized stimuli—that is, stimuli applied to the entire surface of the animal at once, as when they are dropped into some chemical solution or when the vessel containing them is strongly jarred—all the organisms respond by swimming first *backward*. In such a case it is perhaps only the anterior end that actually receives a stimulus.

In a recent paper¹ I have attempted to set forth the bearing of the results gained by a study of the motor reactions of *Paramecium* on the psychology of that animal. The investigations recorded in the present paper require a modification of one of the statements therein made, — if not for *Paramecium*, at least for other infusoria. This is the statement that the direction of motion in the motor reaction has no relation to the localization of the source of stimulus. This statement was based on the reactions of *Paramecium* and other infusoria to localized chemical stimuli, and the reactions of *Spirostomum* to localized mechanical stimuli. These phenomena taken alone seem to justify the statement for *Paramecium* and *Spirostomum*, yet the facts obtained by a study of other infusoria show that the statement cannot be generalized for this group of animals. We have in the infusoria a remarkable transitional stage toward a real perception of the localization of the stimulus, — reaction with regard to such localization so far as motion along the body axis is concerned; a blind reflex without regard to the localization of the stimulus, so far as motion sideways is concerned.

(5) On the whole, the investigation has shown that the motor reaction plan of the infusoria studied is essentially the same as that previously described for *Paramecium*. Until it is clearly shown that some members of these groups react in a manner essentially different from that which I have described, we may assume provisionally that this manner of reaction is characteristic for the Flagellata and Ciliata in general. We may therefore extend provisionally the chief general conclusions drawn from a study of the movements of *Paramecium* to the Flagellata and Ciliata as a whole. The more important of these conclusions may be stated briefly as follows: —

1. The motor reactions to stimuli in the Flagellata and Ciliata take the form of a reflex of definite character, the usual features of which are that the animal moves backward some distance, turns toward a structurally defined side, then moves forward.

2. Different kinds of stimuli do not produce correspondingly different kinds of reaction, but this motor reflex is produced by chemical stimuli of all sorts, by fluids active through their osmotic pressure, by heat, by cold, and by mechanical shock; in fact, by all agents capable of causing a motor reaction. Chemotaxis, tonotaxis, thermotaxis, etc., are therefore not essentially different forms of

¹ JENNINGS: *American journal of psychology*, 1899, x, p. 503.

activity: they are due to the same reflex, merely induced by different agents.

3. The direction of motion throughout this reflex has to only a very limited degree a relation to the localization of the stimulus. The direction of turning has absolutely no relation to such localization, being determined by structural differentiations. Whether motion shall take place backward or forward along the body axis is however to a certain extent determined by the localization of the stimulus.

4. The general effect of this reflex is to take the organism out of the sphere of influence of the agent causing the reaction, and to prevent it from re-entering.

5. Chemotaxis is by no means a passive motion due to attraction or repulsion of the protoplasmic substance by other substances, but is an active movement due to the production of this motor reflex by the chemical agent in question. The organisms leave certain areas vacant ("negative chemotaxis") as a result of the fact that the influences at work in these areas cause this motor reflex. They gather in certain areas ("positive chemotaxis") when the conditions within these areas are *not* such as to cause the motor reflex, while the surrounding influences do cause it. There is then no such thing as direct attraction or repulsion shown by these animals. Corresponding statements may be made for thermotaxis, tonotaxis, etc.

6. The motor reflex through which these reactions are produced is of the same order as the motor reflexes of higher animals, so that there is no reason for holding the reactions of these unicellular organisms to be of an intrinsically different nature from those of higher forms.

7. The behavior of these organisms shows them to occupy an extremely low place in the psychological scale, most of their activities being due to a single reflex.

8. There is evidently no immediate analogy between the reaction movements of these unicellular organisms and the growth movements of higher forms ("tropisms"), so that the phenomena shown by the former do not justify the drawing of direct conclusions concerning the latter.

These conclusions, as well as others of a less general nature, have been developed in detail by the author in previous papers,¹ so that it is not necessary to dwell upon them here.

¹ See, in addition to the papers previously cited, a lecture by the author on "The Behavior of Unicellular Organisms," in the Woods Holl Biological Lectures for 1899.

The investigations above recorded were carried on in the Laboratory of the U. S. Fish Commission for the Biological Survey of the Great Lakes at Put-in-Bay, Ohio, during the summer of 1899. I desire to acknowledge my indebtedness to the officials of the Commission and to Professor Jacob Reighard, Director of the Survey, for their courtesy and assistance throughout the work as well as for permission to publish this paper.

OBSERVATIONS ON THE NITROGENOUS METABOLISM
OF THE CAT, ESPECIALLY ON THE EXCRETION
OF URIC ACID AND ALLANTOIN.

By LAFAYETTE B. MENDEL AND ERNEST W. BROWN, Ph.D.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

THE experiments which form a part of this paper were begun in continuation of a previous investigation in this laboratory on the excretion of kynurenic acid in various animals.¹ Our earlier experience had shown that kynurenic acid is absent from the urine of the cat during both fasting and proteid feeding, — conditions under which this acid is regularly found in the dog. The largely increased excretion of kynurenic acid found in the dog during the increased proteid metabolism caused by phlorhizin administration led us to try similar experiments on the cat. It was reasoned that if kynurenic acid were a normal product of metabolism which is ordinarily destroyed as soon as it is formed in this animal, then by bringing about an unusually marked stimulation of metabolism this product might appear, owing to the temporary inability of the organism to utilize all the intermediary substances formed. Extracts from protocols follow: —

Phlorhizin experiments. — I. A medium sized cat received subcutaneously in four doses 1.5 grams phlorhizin dissolved in dilute sodium carbonate solution. The urine of two days, including the day of injection — 208 c.c. — was examined for kynurenic acid by Capaldi's ² method with negative results.

II. A very large cat received subcutaneously a total of two grams of phlorhizin in two doses with an interval of eighteen hours. The greater portion of the urine of four days — 209 c.c. — was examined for kynurenic acid with negative results. On the two days of largest sugar excretion, the urine contained 10.0 grams of dextrose and 3.92 grams of nitrogen.

These experiments failed to demonstrate any production of kynurenic acid in the cat. We have made additional unsuccessful attempts to find kynurenic acid in the urine of the same animal under condi-

¹ MENDEL and JACKSON: This journal, 1898, ii, p. 1.

² CAPALDI: Zeitschrift für physiol. Chemie, 1897, xxiii, p. 92.

EXPERIMENT I.

Four cats were used in this experiment. The meat fed was chopped lean beef; the thymus was a preparation (Armour's) of the desiccated glands, and was fed mixed with meat.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grams.	grams.
II. 21	450	— ¹	Meat, 450.
22	300	— ¹	" "
23	210	0.112	" " thymus, 25.
24	350	0.215	" " " 50.
25	334	0.291	" " " 100.
26	320	0.108	" 500.
27	190	0.015	" "
28	237	0.012	" "
III. 1	260	0.011	" "
2	170	0.009	None.

¹ Faeces in the urine; a few crystals of uric acid were separated.

tions of fasting and during an abundant diet of both meat and pancreas. There is therefore no modification of our earlier conclusion to be suggested.¹

Experiments on uric acid excretion.—The negative results just recorded induced us to ascertain whether similar differences exist between the organism of the cat and the dog in respect to other important products of metabolism. Our attention has thereby been directed particularly to the excretion of uric acid and allantoin. Regarding the occurrence of uric acid in the urine of the cat few references are to be found in physiological literature. It is reported to be absent frequently from the urine of both cat and dog, having been found regularly only during animal diet and starvation. During a diet poor in proteids it has been observed to disappear.² Since these observations were made before the introduction of the newer methods for the

¹ Cf. MENDEL and JACKSON: *loc. cit.*, p. 27.

² See HUPPERT: Neubauer und Vogel's Analyse des Harns, 10te Auflage, 1898, p. 311.

EXPERIMENT II.

Two cats were employed in this experiment. The food consisted of lean meat alone; meat mixed with desiccated thymus; fresh pancreas.

IS th .	Vol. of Urine.	Uric Acid.	Food.	
	c.c.	grams.	grams.	
III. 10	170	daily average	Meat, 200.	
11	130		" "	
12	117		" "	
13	180		" " thymus, 25	
14	150		" " " "	
15	195		" " " "	
16	155		" "	
17	150		" "	
18			" 250;	
19			" "	
20	112	0.009	" "	
21			" "	
22			" "	
23			" "	
24	170	0.011	Pancreas, ca. 200. ¹	
25	120	0.014	None. ¹	
26	270	0.159	Pancreas, 780.	
27			Meat, 200-250.	
28		daily average	" "	
29	88		" "	
30			" "	
31			" "	
				" "

¹ The cats rejected part of the food and refused to eat.

¹ The cats rejected part of the food and refused to eat.

complete precipitation of uric acid, it may reasonably be questioned whether they possess more than relative value.

The method employed in the present experiments has usually con-

EXPERIMENT III.

Two cats were employed. The diet consisted of the same foods as in the preceding experiment. The pancreas fed was fresh, lean, sheep's pancreas.

1899.	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grams.	grams.
III 12	daily average 86	0.014	Meat, 200-250.
13			" "
14			" "
15			" "
16	205	0.036	Pancreas, 310.
17	190	0.203	" 570.
18	180	0.115	" 411.
19	220	0.100	" 400.
20	daily average 143	0.026	Meat, 250.
21			" "
22			" "
23			" ca. 200; thymus, 25 ¹
24	170	0.036	" little; " (?)
25	101	0.017	" very little (50).

¹ Part of the food rejected on this and the following day.

sisted in feeding several cats in the same cage with various foods, collecting the urine and determining the uric acid present by the Ludwig-Salkowski method. In several series the acid separated has been estimated by titration with permanganate solution instead of weighing. No attempt has been made to collect the urine for sharply defined periods; the animals were allowed water ad libitum and they discharged their urine with considerable regularity. The collections were made daily, and it was found that the results thus obtained with different diets during periods of several days were sufficiently pronounced to afford definite conclusions. Extracts from protocols will be found in Tables I-VII.

EXPERIMENT IV.

Three cats were employed. The diet consisted of lean meat, or fresh sheep's pancreas.

1899	Vol. of Urine.	Uric Acid.	Food.
	cc.	grams.	grams.
III. 31	daily average	0.011	Meat, 250.
IV. 1			" "
2			" "
3			" "
4	lost	—	Pancreas, 300.
5			" 500.
6			" 550.
7			" 550.
8	daily average	0.120	" 300.
9			" "
10			" "
11			" "
12			

The experiments recorded suffice to demonstrate the ready production of uric acid after ingestion of foods rich in nuclein substances, *e. g.*, thymus and pancreas. The persistence of a high uric acid excretion frequently on the day following the feeding of the glands is doubtless in part due to the method employed in collecting the urine (lack of catheterization) rather than to any characteristic after-effect of the diet. The results show so complete an analogy with the similar feeding experiments on man and on the dog,¹ that detailed discussion seems unnecessary here. For the same reason feeding experiments with pure nucleins were omitted.

Experiments on allantoin excretion.—We have been unable to find

¹ For references to the literature on thymus and pancreas feeding see: (man) HUPPERT: *loc. cit.*, p. 313; TH. COHN: *Zeitschrift für physiol. Chemie*, 1898, xxv, p. 509; HOPKINS and HOFE: *Journal of physiology*, 1898, xxiii, p. 271; WEISS: *Zeitschrift für physiol. Chemie*, 1899, xxvii, p. 216; J. ROMÉ: *Journal of physiology*, 1899, xxv, p. 98; TAYLOR: *American journal of medical sciences*, 1899, cxviii, p. 141; (dog) MINKOWSKI: *Archiv f. exper. Pathol. u. Pharmacol.*, 1898, xli, p. 376.

any detailed references to the excretion of allantoin in the cat. Meissner¹ has reported finding allantoin in small quantities in the urine of several cats living on animal diet. It seemed especially desirable to investigate the possibility of allantoin excretion in the cat, since this substance has been detected only rarely in the urine of man, thus giving further evidence of noticeable differences between certain metabolic processes of the dog and some other animals closely related. Quite recently the pronounced excretion of allantoin in the dog has been noted by Minkowski² and by Th. Cohn³ after thymus feeding; similar observations after pancreas feeding are reported by Salkowski.⁴ These results have since repeatedly been verified in this laboratory.

EXPERIMENT V.

Three cats were employed. The pancreas fed was from calves.

1899	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grams.	grams.
V. 14	280	} daily average 0.029	Meat, 500.
15	150		" "
16	120		" "
17	190		" "
18	200		Pancreas, 500.
19	250	0.215	" 700.
20	350	0.156	Meat, 500.
21	300	0.137	" "
22	180	0.087	" 450.
23	180	0.032	" "

Our attention has been directed to the occurrence of allantoin under similar conditions of diet in the cat. In no case has this substance been missed in the urine after thymus or pancreas feeding. Thus, in one of the experiments, the urine obtained after feeding 175 grams of

¹ MEISSNER: *Zeitschrift für rationelle Medicin*, 1868, xxxi, p. 303.

² MINKOWSKI: *Centralblatt für innere Medicin*, 1898, No. 19; *Archiv f. exper. Pathol. u. Pharmacol.*, 1898, xli, p. 376.

³ TH. COHN: *Zeitschrift für physiol. Chemie*, 1898, xxv, p. 507.

⁴ SALKOWSKI: *Centralblatt für die medicinischen Wissenschaften*, 1898, p. 929.

EXPERIMENT VI.

Three cats were fed. In addition to uric acid determinations, total nitrogen was estimated in the urine by the Kjeldahl method; and phosphoric acid by titration with uranium solution. The thymus was a desiccated preparation used in other experiments.

1899.	Vol. of Urine.	Nitrogen.	P ₂ O ₅ .	Uric Acid.	Food.
	c.c.	grams.	grams.	grams.	grams.
IV. 30	120	} daily average	0.801	0.014	Meat, 300.
V. 1	250				" "
2	190				" "
3	360				" " thymus, 100.
4	380	22.99	2.300	0.519	" " " "
5	250	21.27	1.326	0.050	" "
6	150	18.86	1.056	0.015	" "
7	} daily average	18.27	1.014	0.009	" 250.
8					" "

desiccated thymus (Armour's) to four cats was concentrated to a syrup and extracted with alcohol. The alcoholic extracts deposited 1.6 grams of characteristic crystals on standing. After recrystallization, the preparation began to melt at 215° C. and gave the characteristic reaction with furfural. An analysis of the preparation gave 34.99 per cent N; theory, 35.44 per cent N. The crystals were thus identified as allantoin. Again, after feeding about 2½ kilos of fresh pancreas, 2 grams of allantoin were separated in the form of characteristic crystals. After recrystallization they began to melt at 216–217°C. and showed a nitrogen content of 35.12 per cent. Salkowski¹ obtained 3.08 grams of allantoin from the urine of a small dog to which 1¾ kilos of ox-pancreas were fed in five days. It is not uncommon to find crystals of allantoin separating from cat's urine after pancreas feeding, even before the urine is concentrated. In view of Salkowski's experience with dogs,² we have searched for allantoin in the urine of the cat after meat diet, but without success.

Uric acid feeding.—Since it has been demonstrated that uric acid

¹ SALKOWSKI: *loc. cit.*, p. 930.

² SALKOWSKI: *Berichte der deutschen chemischen Gesellschaft*, 1878, xi, p. 500.

ingested, in the case of the dog, is excreted again in part as allantoin,¹ similar experiments were carried out on the cat. Thus, two cats used in previous experiments were fed 6 grams of uric acid mixed with 200 grams of chopped meat. The urine — 360 c.c. — collected on the three succeeding days was concentrated, and about 0.5 gram of allantoin was obtained. In another experiment, in which $4\frac{1}{2}$ grams were fed with 200 grams of lean meat to two cats, 0.3 gram allantoin was obtained. In this respect also, then, the metabolic processes of the cat resemble those of the dog.

Other Experiments.—Quinine is reported by various authors² to diminish the excretion of uric acid in man. We have tried one experiment to determine the influence of this alkaloid on uric acid and allantoin output in the cat after pancreas feeding.

EXPERIMENT VII.

Two cats were employed. The quinine sulphate was administered in small gelatin capsules.

1899	Vol. of Urine.	Uric Acid.	Food.
	c.c.	grams.	grams.
VI. 1	143	0.004	None.
2	90	0.007	Pancreas, 250; Qi sulph. 1.5.
3	200	0.016	" 500; " 2.
4	180	0.027	Meat, 200.
5	340	0.030	" "
6	160	0.011	" "

It will be seen that the increase in uric acid output here observed falls considerably below that seen in other experiments (Exp.'s III, IV, V,) with pancreas feeding. It is scarcely to be assumed that the effects observed are entirely due to deficient digestion and absorption of the material ingested. Allantoin was not detected in this urine after pancreas feeding; this single experiment can, however, scarcely justify any far-reaching conclusions. The same statement

¹ SALKOWSKI: *ibid.*, 1876, ix, p. 719.

² Cf. THOMAS: Neubauer and Vogel's *Analyse des Harns*, 9te Auflage, 1890, p. 246; BOHLAND: *Münchener medicinische Wochenschrift*, 1899, xlv, No. 16; CUSHNY: *Pharmacology and Therapeutics*, 1899, p. 352.

applies to another experiment in which tannic acid was given in relatively large doses to cats, in view of the current statements regarding the excretion of allantoin in man under such conditions.¹ No allantoin was found in the urine, and the uric acid output was apparently not modified in any way.

We have tried to repeat the experiments of Borissow² relative to allantoin excretion in dogs after subcutaneous injections of hydrazine sulphate. The symptoms observed in cats after administration of doses varying from 100 to 150 milligrams resembled in many respects those described for the dog by Borissow. No allantoin was detected; however, the number of our experiments is as yet too small to permit a definite conclusion.

Composition of Cats' Urine.—The lack of data regarding the normal composition of cats' urine has induced us to append the results of a few analyses carried out on urine obtained during various conditions of diet. The figures represent daily averages calculated from the total of several days. Several different animals are represented in the series. Total nitrogen was estimated by the Kjeldahl process; urea nitrogen by the Mörner-Sjöquist method; uric acid according to the Ludwig-Salkowski method; P_2O_5 by titration with uranium solution; SO_3 gravimetrically. Creatinine was found present in cats' urine and separated as the zinc chloride compound.

Average daily excretion for the cat (grams).

Diet.	Total N.	Urea N.	Uric Acid.	P_2O_5 .	SO_3 .
Meat, ca 100;	4.06	2.56	0.009	0.600	0.263
" 85;	4.55	2.67	0.007	0.400	
"	3.52		0.005	0.678	
" 85;	3.38	2.42	0.004	0.694	0.212
"			0.005	0.645	
"			0.008		
None,			0.003	0.610	
"			0.003		

SUMMARY.

1. Kynurenic acid is not excreted by the cat even during the increased proteid metabolism produced by phlorhizin administration.
2. The ingestion of thymus and pancreas tissue causes a marked increase in the uric acid output of the cat, thus corresponding with the similar observations on man and the dog.

¹ Cf. ANSCHÜTZ: v. Richter's *Organische Chemie*, 1897, i, p. 335; also BOLLAND: *loc. cit.*

² BORISSOW: *Zeitschrift für physiol. Chemie*, 1894, xix, p. 499.

3. Allantoin excretion is likewise observed after thymus and pancreas feeding, and after uric acid ingestion. Preliminary experiments have failed to demonstrate allantoin production after administration of hydrazine sulphate.

4. The ordinary daily uric acid output per kilo of body weight in the cat is scarcely smaller than that observed in man and the dog. Creatinine is ordinarily present in cats' urine in noticeable quantity.

DO THE REACTIONS OF THE LOWER ANIMALS
AGAINST INJURY INDICATE PAIN
SENSATIONS?

BY THE LATE PROFESSOR W. W. NORMAN, OF THE UNIVERSITY OF TEXAS.
WITH ADDITIONAL NOTE BY JACQUES LOEB.

BIOLOGISTS are divided as to the correct answer to the above question: some supporting the view that all animals, even the lowest, are capable of suffering pain, and that the reactions of these animals to external stimuli are, or at least may be, the manifest expression of psychical processes resulting from these stimuli. To illustrate: The flesh-fly (*Musca vomitoria*) is according to these authors attracted by the agreeable odor of decaying meat, and being aware of the proper food for its offspring, deposits its eggs on the putrid meat. Likewise, "The flesh-fly deposits its eggs in the flowers of the 'carriion plant' (*Stapelia hirsuta*), the smell of which resembles that of putrid meat and so deceives the fly."¹ The larvæ of these same animals when they have reached their growth and are about ready to pass into the pupa stage find strong light, particularly direct sunlight, disagreeable and try to shun it. *They hate the light.* The moth flies into the flame, being attracted by *curiosity* to get acquainted with the unfamiliar object. The crab caught by its enemy, breaks off its own leg, *hoping* thereby to escape. The angleworm squirms when the boy baits his hook with it, because the rough treatment *hurts* it, and so on.

The supporters of this view ignore the difficulty that confronts them, namely, the only means we possess of studying the psychical processes of the lower animals are the movements themselves, which again are to be explained through psychic processes! One of these authors, Verworn, expresses himself as follows: "It might be questioned whether it is at all possible to reach any conclusion whatever concerning the subjective states of another organism, since they lie external to the investigator. However, the following consideration proves the possibility of arriving at definite conclusions (!) in this direction. We know on the one side from our own subjective experi-

¹ Romanes, *Mental Evolution in Animals*, page 167.

ence our own subjective states, and on the other side the objective expressions that characterize them. We possess here, however, two known quantities. A third known quantity we can get by observing the objective expressions of the psychical processes (!) of the organisms to be studied. Now if we make a proportion between these processes of ourselves and of the organisms under investigation, *i. e.*, if the two be compared with each other, then according to the equation given below, we are in a position to reach a conclusion concerning the subjective processes of any given animal. We have,

$$\frac{x}{a} = \frac{c}{b},$$

$$x = a \cdot \frac{c}{b}$$

in which *a* represents the subjective processes of man, *c* their objective expressions, *b* the objective expressions of the subjective processes of the animal under investigation, and *x* its subjective processes to be found out."¹

This method of Verworn consists then in this, — that one presupposes as already present in the organisms to be investigated that which is to be proved by means of the method, — namely, the existence of psychical processes.

The supporters of the other view seek to analyze the reactions of animals as a necessary consequence of the effect of external stimuli upon them. This method is particularly emphasized by Loeb.² He has demonstrated, for example, that the phenomena of orientation of animals toward light agree in every particular with the phenomena of orientation of plants toward the same source of stimulation. Hence the heliotropic reactions of plants must be referred to curiosity or to some other anthropomorphic process, or else it must be admitted that the phenomena of orientation of animals are to be explained on the same mechanical basis as are those of plants.

Loeb has further proved that instinct and will among lower animals represent rather the sum of the influences of certain external stimuli and internal physiological conditions. Cockroaches do not hide in crevices because there they feel a sense of security against the kitchen-maid, or hold themselves vertically against dark walls

¹ VERWORN: *Psycho-physiologische Protistenstudien*, Jena, 1889.

² LOEB, J.: *Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen*, Würzburg, 1890.

because they particularly enjoy that position, — but because in the first instance the stimuli arising from the contact of a large surface of their body against solid objects exert a quieting influence on them, just as gravity in the second instance tends to make them move about till they come into the vertical position. The flesh-fly is oriented by volatile substances emanating from putrid meat; the same cause leads it to deposit its eggs in the flowers of the carrion plant. The adult larvae of this same animal do not flee from the light because they hate it, but because they are forced to orient themselves in the direction of the rays of light, and in this case to move with the rays from their source, instead of towards it as in the case of moths.

Loeb¹ has further emphasized the fact that consciousness is a function of associative memory (*associatives Gedächtniss*). But memory has thus far been proved with certainty for a limited number of forms. Indeed the facts of brain physiology speak decidedly against the view that phenomena of consciousness are everywhere present in the animal kingdom.

It cannot be denied, however, that certain reactions of lower animals against injury, which in man cause pain, easily lead the inexperienced person to the conclusion that these animals really suffer pain. An earthworm, for example, touched with the point of a needle or otherwise slightly stimulated, may be thrown into violent squirming and jerking motions.

According to Loeb we have no more right in this case to conclude that these motions are due to pain sensations than we have to make a similar conclusion upon the contractions of an isolated frog's muscle when placed in certain salt solutions. I have made a number of experiments which show that this view is correct.²

Experiments on Worms. — *The Earthworm (Allolobophora)*. — If an earthworm be cut in two in the middle only the posterior half shows the squirming and jerking motions which appear to indicate pain. The anterior half crawls away. That had been observed by Friedländer, and Loeb had observed similar differences in the reactions of other similarly treated worms. It would certainly be very strange if only the posterior half of an earthworm should show pain sensations, while the front half, which contains the brain, showed no such reactions. Still more remarkable, however, are the results as one continues with the divi-

¹ LOEB, J.: Archiv f. d. ges. Physiol., 1894, lvi., p. 266.

² Cf. my preliminary paper on the subject, Archiv f. d. ges. Physiol., 1897, lxxvii, p. 137.

sion of the pieces. Let us designate the front half of the divided worm with a and the posterior half b . Now if we cut a in two in the middle, the front piece, which we will designate as a_1 , elongates and creeps forward, while the posterior piece a_2 executes strong jerking and squirming motions. Now if we cut in two the piece b (the posterior half of the worm), the front piece b_1 behaves or reacts as a_1 , and the posterior piece b_2 as a_2 . *We now see that each time the whole animal or either half of it is cut in two the posterior piece makes squirming and jerking motions, while the front piece makes no motions or reactions that could be considered as indicating pain.*

The division of the pieces may be carried even further, and yet the same phenomena are repeated: the posterior halves of a_1 , a_2 , b_1 , b_2 , make the jerking and squirming motions; the front pieces elongate and move forward. The length of the piece naturally modifies the degree of the reactions. With small lively worms, however, I have observed pieces not over five millimetres in length make the characteristic reactions.

If we conclude that the earthworm is capable of feeling pain because of its vehement response to injury, then we must likewise conclude that any isolated piece whatever possesses the same capability. But since it is only the posterior half of any such piece that shows these reactions, we must conclude that it is only the posterior half of the worm or any given piece of the same that feels the pain while the corresponding anterior part has no such pain sensations. But since the front half of any given piece could as easily have been the posterior half of another piece, we must look for another explanation, which must be this, that the nature of the reactions of any given piece of the earthworm is a function of the direction in which the impulse travels. If the impulse travels from the injured spot *anteriorly* the piece of worm elongates; this initiates the normal progressive movements. If, however, the impulse travels from the injured spot *posteriorly* the piece of worm executes the characteristic squirming and jerking motions. In the first case the circular muscles are the first to contract; this causes the elongation of the piece. In the second case the longitudinal muscles are the first to contract; this causes the jerking and winding motions. Why the impulses travelling forwards should always reach the circular muscles first, and those travelling backwards always the longitudinal, I am not able to say.

These reactions of the earthworm are not peculiar to this special mode of application of the stimulus, but are characteristic for any

injury having a clearly limited local application. For instance, if the worm be struck with the back of the scalpel instead of the sharp edge, thus avoiding actually cutting the animal, the reactions are of the same nature. The part of the worm in front of the injured spot elongates and hastens away, dragging with it the posterior portion now squirming and jerking. One can obtain similar reactions by means of chemical and electrical stimuli locally applied.

Cerebratulus. — *Cerebratulus*, a Nemertian worm, is a ribbon-shaped animal reaching a length of eighteen inches or more. Its normal motion in water is an easy undulatory one, not unlike that of a leech. This animal, like *Synapta*, a Holothurian, is difficult to collect as a perfect specimen, as it is apt to break into pieces when disturbed, the wounded place healing over at once and appearing as the normal end of the animal. If while it is swimming, a piece, let us say the posterior third, be cut off with sharp scissors, the part posterior to the cut drops to the bottom, while the anterior end after a brief halt continues its normal swimming motions. This experiment may be repeated in the same manner any number of times with similar result if the piece remaining be long enough to execute the swimming motions. These reactions of *Cerebratulus* had also been observed by Loeb.

Thysanozoon and Planaria. — Loeb's experiments on these animals recited in his article on Brain-physiology of the Worms, page 249, have direct application also to the solution of the question under consideration. He says: "If we cut a *Thysanozoon* in two crosswise with a pair of scissors while the animal is gliding along the surface of the water, the posterior aboral half falls at once as a dead mass to the bottom, while the oral piece which contains the brain creeps quietly on. If the scissors are sharp and the cut be rapidly executed, the behavior of the oral piece shows nothing that indicates a greater excitement, such as we find accompanying phenomena of pain in the case of the higher animals. If the animal be cut in two with a sharp knife while it crawls on a glassplate, we have the same phenomenon. The oral piece creeps on uninfluenced, while the progressive motion of the posterior piece ceases at once. Occasionally, however, the cross-sectioning of the *Thysanozoon* induces more rapid progressive motion. . . . If *Planaria torva* be cut in two crosswise the posterior half, which possesses no brain, creeps on just as lively as the anterior end. . . . Each piece of the animal not too much reduced in size possesses spontaneity."

Podarke obscura. — *Podarke* is a small Annelid, reaching a length

of about 20 mm. When moving on the bottom of a vessel of water it owes its progressive motion to the use of its parapodia aided by a slight serpentine motion of its body. It is an active little worm and reacts readily against any slight mechanical stimulus, — moving rapidly away. For convenience of experimenting with worms, I had a soft pine board slightly excavated to a shallow bowl in which the extent of the movements of the animal could be controlled by the amount of water used. *Podarke* was placed on this board in a few drops of water and very short pieces were cut off from its posterior end by means of a sharp scalpel. The anterior part of the animal remaining after each cut acted as an entire normal animal under the influence of slight mechanical stimulus, — namely, it moved for a short time more rapidly after each repeated stroke of the scalpel. When on the contrary the head end was cut off, the beheaded animal wriggled and jerked about for a brief period, reminding one of the reactions of the similarly treated earthworm. If both the entire animal and the beheaded one be put into dilute formalin, the chemical stimulus calls out rapid swimming motions of the same nature in both cases, but the reaction follows more quickly in the case of the entire animal.

Nereis. — Put a large *Nereis* into the shallow wooden bowl in which is a little water. Now while it is crawling forwards cut off pieces from the posterior end about one inch long, each time with a single stroke of a sharp knife, until but two or three inches of the animal be left. After each cut allow a brief pause to intervene. During this time the head-bearing end has continued to crawl in a normal progressive manner, each stroke of the knife serving as a stimulus to keep the worm crawling, just as a mere touch each time would have done, no irregular movements arising. If the pieces have been cut off in fairly rapid succession we now see the striking phenomenon of the head end crawling forwards, and a half dozen or more pieces squirming and jerking about. The power of feeling pain resides in the posterior pieces! Not every experiment gives the same result, for occasionally the anterior pieces react as the posterior, but in a less degree.

If the two halves of the worm, one possessing the brain, the other not, be chemically stimulated, as by dropping both into a dilute solution of formalin, the irregular reflexes of the two halves are alike.

The Leech (Branchiobdella). — The normal habitat of the leech is water in which it moves about by executing very graceful swimming

motions with its dorso-ventrally flattened body. While on land it changes this method of locomotion and moves like a loop-caterpillar, using the sucking disk at either extremity as the supporting organ.

A leech in a vessel of water is usually found at rest, holding to the wall of the vessel by its anterior sucking disk. If it be freed from its attachment it swims about for a shorter or longer time before attaching itself again. If while swimming about in the vessel it be cut in two in the middle with a pair of sharp scissors, the two halves thus arising continue their progressive motion as if the one worm had suddenly divided into two of its own accord, — the rate and manner of motion slightly modified because of the modified length and form of the new individuals. Neither the anterior nor posterior piece makes an irregular twist or jerk. (Unlike the earthworm, not even the posterior half (!) becomes excited because of the pain sensations.) Occasionally both halves come to a momentary pause at the instant of the experiment, then each continues as an independent organism.

If leeches be transferred from water at 22° C. (the temperature of the laboratory) to water heated to 40° C. the increased temperature proves to be a very effective stimulus, — the animals executing rapid, and at first more or less irregular, swimming motions. If they be placed in water heated to 50° C. they sink relaxed and motionless to the bottom. The temperature of 40° C. that excites the vigorous motions is but little above that of our blood with which the animal at one time can fill itself for a several months' supply of food. No one could entertain the idea of pain in the last act. It is about as rational to think of pain as being the exciting cause of the movements in the former instance.

If the posterior piece or a piece sufficiently long taken from the middle be held between the blades of the forceps or held down upon a solid object the stimuli give rise to strong irregular reflex motions which the inexperienced might again regard as due to pain. They may indeed be much more pronounced than if the whole animal were so treated.

The Echinodermata. — Although the animals of this subkingdom have definitely differentiated nervous and muscular systems, yet the peculiar anatomical plan of the organs of locomotion and the resulting excessively slow movements of the animals make it *a priori* improbable that they would furnish any evidence in favor of the theory of pain sensations. Romanes, however, places with a question mark

the Echinodermata in the list of animals having intelligence. He further states with emphasis that when being injured they move away from the source of the injury.¹

The Starfish (Asterias) and Brittle-star (Ophiura).—When a starfish is lifted up from the surface along which it is creeping it makes many miscellaneous haphazard movements with its tube-feet, and slowly twists its arms into different positions. If placed on its aboral surface it makes the same awkward movements, but gradually rights its position. The various movements of the tube-feet and the arms made by the animal when held in the hand or placed on its aboral surface are as indicative of discomfort as the severest injury that can be made upon it.

As every one knows a starfish may be cut into pieces or otherwise mutilated with no other reaction than a temporary contraction of the tube-feet. Further, the pieces, if not too short, soon begin the same slow easy motion that characterizes that of the entire animal, as if no injury had been done.

The brittle-star moves by using its very slender arms as levers, dragging one or two (not being used) in the rear. If one of the arms in use be pinched or the end clipped off, or otherwise stimulated, the only response the animal makes is a change usually very slight in the direction of its progressive motion.

If when a starfish is crawling it be struck on one arm the jar causes a temporary withdrawal of the tube-feet into the ambulacral furrow, thus allowing the other arms to change the direction of the progressive movement. Indeed, one may cause the animal to suspend its motion in all directions by successively tapping the different arms. Romanes's remark that the Echinodermata travel from the source of injury was not based on sufficient observation.

Crustacea.—We shall now consider the Crustaceans. In the case of the animals of this group,—as the crayfish, the lobster, and the crab,—their hard exoskeleton prevents the easy application of stimuli in a well regulated manner.

The Hermit Crab (Eupagurus).—The exoskeleton of the body, particularly the abdomen, of the hermit crab in contrast to that of other crustaceans is soft, the lime salts being absent. If one of these animals be freed from its appropriated house (some snail shell) and either the reduced abdominal appendages or the abdomen itself be pinched with forceps, the animal responds at once with various

¹ ROMANES: Jelly-fish, Star-fish, and Sea-urchins, p. 282.

motions, especially of the thoracic appendages, — the chief organs of locomotion. Now if the animal be suspended, allowing the posterior end to hang downwards, the abdomen may be cut in two with a pair of scissors and the part posterior to the cut fall entirely away, with but very slight response on the part of the movable organs of the main part of the animal remaining. Indeed the experiment was repeatedly made so successfully that not a motion followed. Yet, when the animal was put down, it moved about in its usual way, not at all modified by the loss of much of its abdomen.

Autotomy practised by the crabs. — If one examines a number of crabs he will be struck by the presence of appendages much smaller than the normal sized ones; these are in the process of regeneration. One may observe the occasion for this regeneration if he holds a crab fast and presses one of the legs vigorously or otherwise injures it, as by clipping off with a pair of scissors pieces from the distal portion. He will now see the injured leg or even one or more of the other legs drop off. On one occasion I observed Dr. Loeb take up a spider crab that had just moulted in the aquarium of the Woods Holl Laboratory, and holding the animal by his left hand, all the ten legs being free, cut off with scissors the free end of each of the ten legs, the animal reacting each time to the insult by snapping off the entire leg, until all ten were off. Shall we accept an anthropomorphic explanation for this peculiar action on the part of the crab: that the animal is aware of danger and seeks thereby to escape, or that it is the result of excitement due to pain sensations caused by the injury? Neither explanation would be satisfactory. Fredericq¹ has proved that the reaction is a purely reflex one, and that the crab can still break off its legs after complete removal of its brain.

One may say in general of the Crustacea that gently touching an antenna or a leg or even making a motion of the hand before the eyes may call forth a muscular reaction as vigorous and varied as that produced by a very severe injury.

*The Horseshoe Crab (*Limulus polyphemus*).* — The horseshoe crab makes progressive motion either by crawling along at the bottom of the water by use of its thoracic legs, or swimming chiefly by means of its abdominal appendages, to which the gills are attached. Whether the animal is in motion or at rest, the gills are kept usually in rhythmical motion, thus keeping up a current of water for the process of respiration.

¹ FREDERICQ, L.: *Archives de biologie*, 1892, xii, p. 169.

If a *Limulus* be placed in a dish of water with its ventral surface uppermost, it soon begins its normal respiratory movements, — namely, the to-and-fro motion of its swimmerets. Now touch the animal gently, the swimmerets cease moving and the abdomen is flexed ventrally toward the mouth. After a few seconds the abdomen assumes its former position and the swimmerets resume their respiratory motions. If instead of a mere touch a severe injury with scalpel or scissors be made, what reaction then follows? The same. If four or five thoracic appendages be cut off at one stroke, the rhythmical respiratory movements cease, the abdomen is flexed ventrally, and a pause of a few seconds ensues. After the pause the unfolding follows and then the normal rhythm of the respiratory movements. I found I could make deep cuts into the body and get the same quiet succession of events, — cessation of respiratory movements, folding of abdomen ventrally, pause; unfolding of abdomen, beginning of respiratory movements. Indeed, I could hold the animal in my hand and cut off with strong scissors the posterior half of the abdomen with the same result.

These experiments were much varied, — at one time cutting off several appendages at a single stroke, at another making deep wounds into the thorax, again slicing off portions of the abdomen, or even cutting away its posterior half. The reactions from these various injuries offered no more evidence that the animal experienced pain from them than from those produced by merely touching it.

Myriapoda. — *Centipedes and Millipedes.* — Centipedes and Millipedes, like the worms, are well adapted to experiments of merotomy because of their elongated, slender form, and a pair (in the case of the Millipedes two pairs) of appendages to each segment of the body.

Geophilus. — *Geophilus* is a small, exceedingly elongated centipede, the number of segments in the body reaching almost a hundred. It is the liveliest centipede that I know. Its favorite habitat is beneath the bark of fallen trees in moist woods. When disturbed in this location it runs nimbly about till it finds a crevice, into which it creeps or buries itself in the loose material of the decaying log. When in an open place, as in a dish, one may by merely touching an antenna or appendage, or even any part of the body, start the animal off at a rapid rate. It is irritable in a very high degree. If it be held fast by placing the blunt end of a pencil or scalpel on the middle of its body the reactions of the two halves of the body are

different: the front portion is much more active, — turning, twisting about, and biting at the object which makes it a prisoner, while the posterior portion attempts to pull itself away, the legs working together all pulling toward the posterior tip of the animal. As soon, however, as the weight is removed the centipede runs away, all the movements properly co-ordinated.

If it be cut in two in the middle both pieces immediately after the injury react essentially alike, — they run away, the anterior half always going forwards, the posterior half at first usually backwards. Any piece not too short (four or five segments) can execute progressive movements.

The reactions of a decapitated *Geophilus* to mechanical stimuli are strikingly like those of the entire animal: a slight touch or stimulus causes a sudden start, the increased rate of speed decreasing usually more rapidly than in the entire animal. If one is seeking, however, to find reactions that may be comparable to those of higher animals which are unquestionably accompanied with pain-sensations, they are best shown by the posterior part of the animal, which is cut off from the rest of the body.

Scoleopendra. — A *Scoleopendra* about six cm. long was placed in a vessel containing water on the surface of which it remained. The object of placing the animal on the surface of the water was to allow it the fullest freedom to execute any irregular motions when experimented upon. A piece of about four segments was then cut off from the posterior end of the animal. It reacted to the injury by making several successive to left and right jerking of the body, quite like that of the posterior part of the earthworm when similarly treated, — such movements as convince the inexperienced that the injury is a painful one. The value of this particular experiment toward giving an affirmative answer to the question put as the subject of this article was negated by the reactions of the same animal which took place on making a similar experiment at its head-end, — namely, a repetition of those produced when the posterior part of the animal was cut off. A piece cut off the second time from the posterior end of the animal now beheaded gave reactions of the same nature, but in a less degree.

Lithobius. — *Lithobius* differed in its reactions to injury from the other centipedes experimented upon in that when decapitated it lost all or almost all power of progressive motion. Likewise the reflex actions of this animal deprived of its brain are very weak.

Millipedes (Julus, Polydesmus).—The animals of this group are slow moving, and far less irritable than the centipedes. When the head is removed they still possess the power of co-ordinated progressive movement, but in a very limited degree.

If a normal *Julus* or *Polydesmus* be placed on a solid object as a wooden table and allowed to begin moving away, one can cause severe injury to the animal without interrupting its progressive motion. While it is thus crawling we can clip off pieces from the posterior extremity with a scalpel without exciting the animal into a more rapid pace or into any irregular jerking motions of the body.

Insecta.—Dr. Hargitt (Syracuse University) informs me that he chanced several years ago to observe a dirt dauber (a *Vespa*) lapping at a liquid, and incidentally clipped off its abdomen with a pair of scissors. The animal was not disturbed by the performance but continued to eat while the liquid flowed out at the shortened posterior end of the body.

Bethe in his excellent article on the Comparative Investigations of the Functions of the Central Nervous System of the Arthropods writes: "I have cut off the entire abdomen of bees and then placed them at honey which they sucked unceasingly for more than an hour. Indeed, while the bee was sitting on my hand sucking honey, I suddenly cut off the abdomen. It straightened up for a moment, then sucked quietly on. Whether by such results one can speak of pain or indeed of sensations appears to me doubtful."

Dragon Flies—In addition to confirming the above observations I made experiments of a similar nature on dragon flies, which I held in my hand while I clipped off pieces from the abdomen, without, in some instances, the animal making any change of position at all.

Acridium americanum.—If the grasshopper be held by the wings, thus allowing its body and legs to be free, and one of its legs, let us say one on the prothoracic segment, be cut in two, the animal reacts to the stimulus either not at all or by making a few twitches or slight motions, particularly of the leg of the same body segment. Now cut off the head at the neck, thus removing all of the central nervous system in front of the first thoracic ganglion; then clip off part of the remaining stub or prolong the pressure with the scissors or pair of forceps. Instead of getting a very mild reaction the legs are thrown into strong rapid movements, the state of the muscles approaching that of a tetanus. Here again if we take the reactions to injury as a known factor in the equation then the

grasshopper still in possession of its head does not suffer much in comparison to that which follows from the same degree of injury in case the head is cut off.

The Vertebrata. — We come lastly to the Vertebrates, — the one group that has its nervous system built on the same anatomical plan as that of man. That the higher vertebrates — mammals and birds — have memory and are capable of limited intelligence needs no consideration. The mental capacity of the lower vertebrates is however very limited, and in the lowest, including the fishes, the possession of any mental capacity whatever becomes a question.

Sharks (Squalus). — The functions of the different parts of the internal ear have been the subject of many investigations. The fact that these organs are so easily reached in the case of the Elasmobranch fishes, being enclosed within cartilaginous capsules only, has made them standard animals for vivisection experiments in this line. Dr. Lyon's investigations on the functions of the internal ear made at the Marine Biological Laboratory during the summer of 1898 gave me opportunity to make observations on the reactions of these animals during his experiments.

The fish will rest quietly on the operating board if water obtained by means of a rubber tube placed in the fish's mouth and connected with the water supply is allowed to circulate freely through the gills.

The exposure of the semicircular canals, their ampullae, the auditory nerve, etc., is made by use of a scalpel, and is a tedious process. The strokes made from beginning to end of the operation are many. The reactions of the animals are usually so slight that a bystander would hardly be aware that a perfectly live animal lay before him. Indeed one can make stroke after stroke without the slightest motion of body or fin.

The Flounder (Pseudopleuronectes). — The anatomy of this animal is peculiar because of its excessively compressed body and its head being twisted through an angle of 90 degrees. It can therefore be laid on its assumed ventral side and the head have the same position in space as that of other fishes when placed in their normal ventral position. Because of the excessive flatness of the animal it is an exceedingly convenient form for giving a stable position for the experiments. After observing Dr. Lyon operate on a number of these animals on the board to which they were made fast by means of a piece of net, and noting a lack of any apparent effort to escape from the operator's knife, I suggested that the animal did not need

to be held down. A large specimen was placed on the table and quieted by circulating water through the gills. The net was then at my request left off so that the fish might be free to give full expression by motion of its fins, tail, or of its whole body to any pain which it might feel. The first stroke of the knife slit the skin open about two inches. The fish did not flop off the table; it did not strike with its tail, or make any motions with its fins. It lay quietly and allowed the operator to whittle away the hard bony capsule enclosing the organs of the internal ear. If, however, by some mischance the rubber tube supplying water for respiration fell out of the fish's mouth it at once became restless, and required to be forcibly held down.

ADDITIONAL NOTE BY JACQUES LOEB.

This paper was to have been the Doctor's thesis of my friend Norman. It is completed only as far as the enumeration of experiments is concerned. He intended to write the last chapter in Woods Holl last summer, but died from typhoid fever shortly after his arrival. I have published his paper with practically no alterations and I do not think that I should add the missing chapter. But having seen most of his experiments I may be permitted to point out the two chief results of his investigation.

1. In a great number — perhaps the majority — of lower animals injuries cause no reaction which might be interpreted as the expression of pain sensations.

2. In the limited number of cases where injury is followed by motions which have been considered as the expression of pain sensations (as in the case of worms) a closer analysis shows that this interpretation is unjustified. Only the part behind the place of injury shows such reactions while the part in front of the injury shows nothing of the kind.

In Norman Physiology has lost a powerful and independent worker. His paper opens a new field of research, and his investigations into the reaction of the earthworm upon injury will live in Physiology as classic experiments.

ON THE OCCURRENCE OF IODINE IN THE THYMUS AND THYROID GLANDS.

By LAFAYETTE B. MENDEL.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

THE announcement of Baumann's discovery of iodine as a normal constituent of the thyroid gland, directed attention to the possible occurrence of this element in other organs of the body. For the most part, the investigations in this direction have yielded negative results. In the case of the ovaries, the occurrence of iodine in very minute quantities has been reported by several investigators.¹ The presence of minimal quantities of iodine in the hypophysis has been asserted by some writers,² while others³ have failed to detect it. In the adrenals,⁴ spleen,⁴ and salivary glands,⁵ also, iodine has been detected. When the almost constant presence of iodine compounds in the thyroids of fully developed animals is considered, it cannot seem remarkable that iodine in some form should pass into the circulation and occasionally be found in various organs. In all the instances cited, however, the quantities actually found have been so small that renewed investigation is needed before any serious significance can be attached to existing statements. That iodine is by no means a constant constituent of some of these glands, my own experience has shown; thus I have examined relatively large quantities of ovarian substance (pig's) and salivary gland (ox's) without being able to detect this element.

In his earlier experience, Baumann⁶ was unable to detect iodine in the thymus gland; later, however, in 200 grams of fresh thymus

¹ BARELL: *Chemisches Centralblatt*, 1897, i, p. 608; SEYDA: *ibid.*, 1897, ii, p. 806; LANZ: *Berliner klinische Wochenschrift*, 1898, pp. 371-372.

² SCHNITZLER und EWALD: *Chemisches Centralblatt*, 1896, ii, p. 548; H. G. WELLS: *Journal American medical association*, October-November, 1897. Reprint p. 57.

³ BAUMANN: *Münchener medicinische Wochenschrift*, 1896, No. 14; v. RO-SITZKY: *Wiener klinische Wochenschrift*, 1897, pp. 823-824.

⁴ BARELL: *loc. cit.*

⁵ CUNNINGHAM: *Journal of experimental medicine*, 1898, iii, p. 231 note.

⁶ BAUMANN: *Zeitschrift für physiol. Chemie*, 1895, xxi, p. 325.

glands of the calf, he succeeded in detecting $\frac{1}{10}$ - $\frac{1}{15}$ mgr. iodine.¹ Considering this result in connection with the observations of Mikulicz² on the efficiency of thymus feeding in goitre, Baumann regarded it as probable that the thymus contains iodine in the form of thyriodine (iodothylin), although in much smaller quantity than in the case of the thyroid. Further observations on the thymus scarcely exist. Cunningham³ has repeatedly tested the thymus for iodine without success, and suggests "that possibly accessory thyroid bodies were present in the thymus examined by Baumann." H. G. Wells analyzed the thymus glands of four children. "In one from a four months old child, and in one from a still-born infant, slight traces of iodine could be detected. In the other two, which were both from still-born infants, no traces whatever could be detected, even when three grams of the dried glands were examined at one time."⁴

The writer's attention was first directed more closely to this subject through the examination of a rather unusual accessory thyroid found in the thoracic cavity in a well defined case of acromegaly.⁵ In this case the thyroid proper, which was enlarged and weighed 101 grams, contained a relatively small amount of iodine, in correspondence with the histological findings on examination of the gland. Thus there was a marked increase in the connective tissue as compared with a normal gland; while the colloid material was decreased. The later observations of Hutchison⁶ and Oswald⁷ have shown, however, that the iodine content of the thyroid is particularly associated with the presence of colloid matter. The large accessory gland found in the median line high up in the thoracic cavity, just above the upper end of the sternum, weighed $36\frac{1}{2}$ grams. From its location it was at first regarded as thymus. It showed a distinct "division into a cortical and a medullary portion, the latter constituting somewhat more than half the diameter of the entire body, and being made up of fibrous tissue. The cortical portion resembles that of developing thyroid tissue as described by Halsted⁸

¹ BAUMANN: Münchner medicinische Wochenschrift, 1896, No. 14.

² MIKULICZ: Berliner klinische Wochenschrift, 1895, No. 16.

³ CUNNINGHAM: Journal of experimental medicine, 1898, iii, p. 231 note.

⁴ WELLS, H. G.: *loc. cit.*

⁵ OSBORNE, O. T.: Transactions of the Association of American Physicians, 1897, p. 262.

⁶ HUTCHISON: Journal of physiology, 1896, xx, p. 474.

⁷ OSWALD: Zeitschrift für physiol. Chemie, 1897, xxiii, p. 310.

⁸ HALSTED: Johns Hopkins hospital reports, i.

as being found in dogs after partial extirpation of the thyroid gland." In the central portion no iodine could be detected; in the cortical portion, within the spaces of which colloid matter was observed, no less than six milligrams of iodine were found. It may be added that the patient had undergone no previous treatment involving the use of iodine compounds, which are well known to increase the iodine content of the thyroid.

The preceding details have been recorded not alone because of the unusual conditions observed, but particularly because they emphasize the care which becomes necessary in the removal of thymus tissue, to avoid contamination with possible accessory thyroids. In the parathyroids of the dog and rabbit Gley¹ has found a relatively greater content of iodine than in the thyroids of the same animals.

The data to be presented in this paper include analyses of both human and animal glands.² In searching for iodine, the finely-divided, dried gland-substance was fused in a nickel crucible with sodium hydrate and potassium nitrate, and this process—together with the succeeding colorimetric estimation of the iodine in the fusion products—was carried out according to the directions of Oswald.³ Silver crucibles were never employed, since they may occasion loss of iodine owing to the formation of insoluble silver iodide. The reagents used were repeatedly examined for iodine with negative results. In testing the efficiency of the method when applied to large quantities of material, I have found no difficulty in recovering at least 3% mgr. iodine when 2% mgr. iodine as KI were added to about 15 grams of dried serum-proteids or fibrin.

A summary of a few analyses of thymus and thyroid glands from infants follow. The weight of each thymus varied from three quarters to three grams, dried; the thyroids were somewhat smaller.

The thymus and thyroid glands of four infants at full term were examined for iodine with negative results. In the same glands from two infants about 24 days old, the thymus contained no iodine, while the thyroid contained 0.07 mgr. iodine. The thymus from the following individuals likewise showed no iodine whatsoever.

¹ GLEY: *Comptes rendus*, cxxv, p. 312.

² Grateful acknowledgment is made to Professor H. B. Ferris of the Yale Medical School and to Dr. Joseph Roby of Rochester, who have furnished suitable material; and particularly to Armour and Company of Chicago, who have generously prepared various gland products for me.

³ OSWALD: *Zeitschrift für physiol. Chemie*, 1897, xxiii, p. 275.

Infant	26	days	old	cause of death:	gastritis.
48	"	"	"	"	inanutition.
2	months	"	"	"	ileo-colitis.
4½	"	"	"	"	pneumonia.
6	"	"	"	"	"
8	"	"	"	"	colitis.
20	"	"	"	"	ileo-colitis.

As regards the thyroid, these results correspond with the observations of Baumann¹ and of Miwa and Stoltzner,² who likewise examined the thyroids of young infants without finding iodine in the great majority of cases. H. G. Wells,³ however, has reported the finding of traces of iodine in thyroids of infants at full term. With reference to the thymus it is evident that the absence of iodine is noticeable even in older individuals in which a considerable accumulation of iodine in the thyroid has already taken place. I have made similar observations in the case of a dog of ten kilos. The thyroids, weighing 550 mgr. air dry, contained about 0.25 mgr. iodine, while no iodine could be detected in the large thymus weighing over two grams, air dry.

Although it may be questioned whether the minute traces of iodine found by Baumann on analysis of a very large quantity (200 grams) of thymus deserve the physiological significance frequently attributed to this single observation, I have repeated these experiments with large quantities of thymus. The fresh glands (calves') were dissected very carefully, and after treatment with absolute alcohol and ether were tested by the method already referred to. Thus in one experiment 213 grams of fresh thymus (yielding 39 grams dry substance) were examined with negative results; similar experiments were made with varying quantities of substance, but in no case could iodine be detected in this material. In two samples of desiccated thymus carefully prepared for me by Armour and Company, I have likewise been unable to find a trace of iodine, even when large quantities were fused: while I have not failed to detect very small quantities of iodine added to equally large amounts of the same desiccated material.

In two samples of commercial thymus preparations, however, I have detected traces of iodine. Nine grams of one of these, labelled

¹ BAUMANN: *Zeitschrift für physiol. Chemie*, 1896, xxii, p. 11.

² MIWA und STÖLTZNER: *Jahrbuch für Kinderheilkunde*, 1897, xlv, p. 87.

³ H. G. WELLS: *loc. cit.*, reprint, p. 25.

"Desiccated thymus containing 50 per cent sugar of milk," showed a faint trace of iodine; another product "Desiccated thymus of the calf," repeatedly yielded about 0.07 mgr. iodine in twelve grams of the substance. In these cases the iodine found presumably was attributable to contaminating materials. Additional weight is lent to this view by the fact that of three commercial thymus preparations from the same source, only one showed traces of iodine; while one of the two iodine-free products had been prepared at my suggestion with particular care to avoid contamination with accessory thyroid bodies.

From the foregoing evidence it seems probable that iodine is not a normal constituent of the thymus and that Baumann's detection of that element in the gland was due to admixture of thyroïdal tissue. Finally, the feeding experiment of Baumann,¹ in which the thyroids of a small dog ingesting 26 pounds of thymus in sixteen days were found to contain 1.4 mgr. of iodine, loses the significance attributed to it, in view of the later experiments of Roos.² The latter investigator found that dogs frequently retain a considerable store of iodine in their thyroids even after six weeks' feeding with meat,—a diet free from iodine. The results of a single brief feeding experiment with thymus can therefore no longer be regarded as a confirmation of the existence of iodine in the gland fed.

The favorable results which have been reported for the thymus method of treatment in both true and exophthalmic goitre have been confirmed more recently through the further observations by Reinbach³ in Mikulicz's clinic. The success of thymus therapy indicates, as Reinbach points out, that the peculiar action of thyroid preparations in reducing goitre is nothing specific for the thyroid. Furthermore Cunningham⁴ has demonstrated that thymus tissue yields substances equally as capable as thyroid extractives of palliating the acute cachexia in thyroidectomized dogs. That these substances do not contain iodine is pointed out by Cunningham and is particularly emphasized by my own experiments.

What light do these experiments throw upon the significance of the iodine content of the thyroid? It seems to the writer that a

¹ BAUMANN: *Zeitschrift für physiol. Chemie*, 1896, xxii, pp. 14-15.

² ROOS: *Zeitschrift für physiol. Chemie*, 1899, xxviii, pp. 43-44.

³ REINBACH: Abstract in *Centralblatt für die medicinischen Wissenschaften*, 1899, p. 276.

⁴ CUNNINGHAM: *Journal of experimental medicine*, 1898, iii, p. 225.

variety of evidence leads to the conclusion early announced by Hutchison,¹ that the physiological activity of thyroid preparations is due to substances associated with the iodine, rather than to that element itself. This, at least, applies to the ordinary action of thyroid feeding in goitre. Two reasons for this conclusion may briefly be stated. First, the thymus, which shows marked similarity to the thyroid in its effect on goitre, contains no iodine. Second, the proportion of iodine in the colloid matter may be artificially increased to even ten times the normal amount without occasioning any increase in the activity of the preparation;² while artificially iodized proteids show relatively little action. In this connection the recent work of Roos³ deserves attention. He found that the effects of equal quantities of dried thyroid in increasing the nitrogenous metabolism of dogs were apparently proportionate to the content of iodine in the glandular tissue fed. Equally varying was the efficacy of the different preparations in reducing the size of parenchymatous goitres. These facts, however, by no means compel the conclusion that the characteristic action is due to the iodine present; with equal probability, we may assume corresponding variations in the accompanying active groups to which the iodine is perhaps attached merely as a factor of secondary importance. Under this interpretation of the facts known, it is not necessary to follow Roos⁴ in attributing the favorable action of thymus to the minute traces of iodine occasionally found in preparations of that gland.

SUMMARY.

1. The accessory thyroids in man may contain both relatively and absolutely more iodine than the thyroid proper of the same individual.
2. The observations that the thyroids of newly-born children contain no iodine are confirmed.
3. There is no satisfactory evidence to show that the carefully isolated thymus of man or animals contains iodine. Traces found by other observers were presumably due to adherent thyroidal tissue.

¹ HUTCHISON: *Journal of physiology*, 1896, xx, p. 494; cf. also REINBACH: *loc. cit.*

² HUTCHISON: *Journal of physiology*, 1898, xxiii, p. 178.

³ ROOS: *Zeitschrift für physiol. Chemie*, 1899, xxviii, p. 40; cf. also OSWALD: *ibid.*, 1899, xxvii, p. 40.

⁴ ROOS: *Zeitschrift für physiol. Chemie*, 1899, xxviii, pp. 50-51.

THE EFFECTS OF IONS UPON THE AGGREGATION OF FLAGELLATED INFUSORIA.

BY WALTER E. GARREY.

[From the Hull Physiological Laboratory of the University of Chicago.]

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I. THE AGGREGATION OF INFUSORIA, HOW PRODUCED.

Introduction.—This investigation was undertaken at Professor Loeb's request and under his direction, with the object of determining to what extent the gathering of infusoria subjected to the influence of solutions of electrolytes was determined by ions, and with the hope of establishing an analogy between the effects of chemicals and the effects of other stimuli upon the motions of these organisms.

Many writers have failed to recognize the fact that gatherings of motile forms subjected to a given stimulus are not always due to the same effect of the stimulus. Dr. Loeb has shown that light may cause gatherings of animals in two ways:—

I. By a reaction of the animal to *sudden changes in the intensity of illumination*, effecting gatherings in the less intensely illuminated area. Such animals Loeb calls "*Unterschiedsempfindlich*,"¹ photokinetic.²

¹ LOEB, J.: *Archiv f. d. ges. Physiol.*, 1893, liv, p. 81.

² The term "photokinesis" (photokinetic) does not express adequately the meaning of the term "*Unterschiedsempfindlichkeit*," but expresses the essential feature of the

II. By an orientation of the animal such that its axis of symmetry coincides with the direction of the rays of light, with a consequent motion to or from the source of light. Such animals Loeb calls "heliotropic."

If a vessel containing various forms of animals representing the above mentioned forms of sensitiveness to light be placed before a

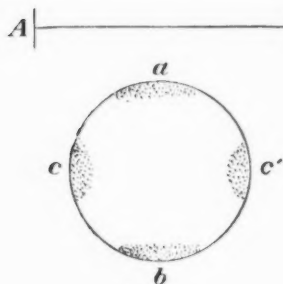


FIGURE 1.

window (Fig. 1, A-B), it soon becomes evident that there is a gathering of certain forms on the side nearest the window (Fig. 1, a). Such forms are said to be *positively* heliotropic (e. g. plant lice). Other forms, which are *negatively* heliotropic (e. g. larvæ of the fly), gather on the side farthest from the window (Fig. 1, b).

Still other forms gather along the sides of the dish where the intensity of illumination is least, e. g. fresh-water planarians (Fig. 1, c and c'). These forms are photokinetic (*Unterschiedsempfindlich*). They become restless if the intensity of the light is suddenly increased, while they become quiet ("fall asleep") if the intensity is suddenly decreased. If they creep about and come into an area where the intensity of the light is comparatively small, they become quiet. Hence those places where the light is a comparative minimum act like traps in which the planarians are caught, and consequently gatherings take place in these less intensely illuminated areas. Besides planarians, earthworms and many other animals show this reaction.

Loeb has pointed out that *every true tropism is due to an orientation* of the organisms. In heliotropism the organism is so oriented that its axis or plane of symmetry coincides with the direction of the rays and symmetrical points on the surface of the body are struck by the light rays at the same angle. The direction of the movement is determined by the direction of the rays of light. In galvanotropism the current curves, that is, the path of migration of the ions, determines the direction of movement.

phenomenon to be described. Davenport proposes the term photopathy, which does not express the increase of motion, and in addition is suggestive of suffering. As a substitute for the phrase "*Unterschiedsempfindlichkeit* to chemicals" I have introduced the term "chemokinesis" and call organisms showing this reaction "chemokinetic."

In chemotropism Loeb introduces the conception of lines of diffusion, *e. g.* the lines along which the diffusing molecules or ions move from a centre of diffusion. The lines of diffusion play the same rôle in chemotropism that the rays of light do in heliotropism or the current curves in galvanotropism.

Loeb expresses the possibility of a perfect analogy of chemotropism with the two other forms of tropism, in these words: "The essence of chemotropic orientation would then consist in the animals placing themselves in such a position that symmetrical points on the surface of the body are cut by the diffusion lines at the same angle. Thus symmetrical muscles are in the same state of tension."¹

If we wish to analyze the reactions of animals to chemicals, we must answer the following questions: Is there a *chemokinesis*? Is there a true chemotropism?

This investigation resulted in an affirmative answer to these questions, and in an establishment of the analogy between the effects of

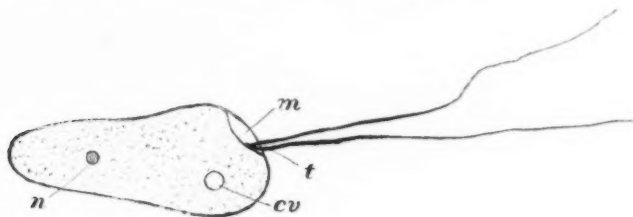


FIGURE 2.

chemicals and the effects of other stimuli which cause gatherings of infusoria. Gatherings take place which are *due to true chemotropism*. The organisms move toward the centre of diffusion of certain chemicals (*e. g.*, weak lactic acid) as if orienting themselves to radially disposed lines; lines which may represent the paths of diffusing molecules or ions. Furthermore, other gatherings of infusoria occur which are *not due to such an orientation*, but to an effect of a change in the concentration of the chemical used (*e. g.*, an inorganic acid) to *chemokinesis*, a reaction completely analogous to photokinesis. A more detailed account of the two kinds of reaction of the infusoria will follow the description of the organism used and of the method employed.

The organism. — *Chilomonas*, the organism chosen for this investi-

¹ LOEB, J.: *Archiv f. d. ges. Physiol.*, 1897, lxxi, p. 442.

gation, shows most marked reaction to chemicals in solution. The cultures were obtained by soaking ordinary peat moss obtained from the Lake Superior region. The single individual is not visible to the naked eye. Under the microscope it presents somewhat the appearance of a diminutive pear, at the broad (anterior) end of which two flagella, twice the length of the body, may be seen (Fig. 2). At the base of these flagella is a funnel-shaped depression, the mouth (*m*). A single contractile vacuole (*cv*) is situated near the mouth. In the posterior half may be found the nucleus (*n*). The whole body presents a coarsely granular appearance.

The method.—The determination of the exact nature of the reactions of the organism was found impossible by the methods heretofore employed, *i. e.*, by introducing into some of the culture capillary

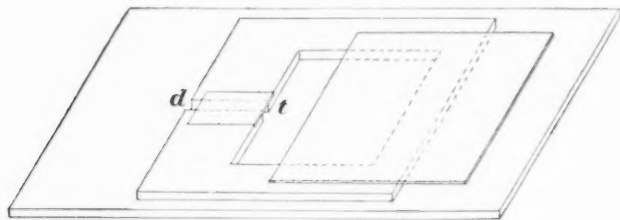


FIGURE 3.

tubes containing the solution to be tested, or by introducing drops of the solution. The device shown in Fig. 3 was used throughout this investigation, and gave most satisfactory results. It consists of a chamber about 1 or $1\frac{1}{2}$ mm. deep, made by fastening hard rubber to a glass slide with sealing-wax. An opening $1\frac{1}{2}$ mm. wide is left in one side of the border about this chamber. This opening is covered by a piece of cover-glass countersunk flush with the surface. The tube thus formed, which will be called the diffusion tube (Fig. 3, *dt*), can be filled by capillarity with the solution to be tested. A cover-glass is pushed over the chamber from the side opposite this tube, and the chamber is filled with the culture as rapidly as it is covered. The culture and the solution in the tube come in contact at the instant the chamber is full and covered.

At the instant of contact between the solution in the tube and the culture in the chamber, the solution will begin to diffuse. The behavior of the organism under the influence of the solution may then

be studied.¹ Let us consider the phenomena in some such cases, noting some details of the reactions which serve to support the assertion already made as to the analogies existing between reactions of infusoria toward chemicals and toward other forms of stimulation. From this study we may also obtain the criteria for the determination of the effects of various ions.

Effects of hydrochloric acid.—If a solution of hydrochloric acid be the solution in the diffusion tube, and the chamber be filled with *Chilomonas* culture, it is noticed that the organisms leave the area

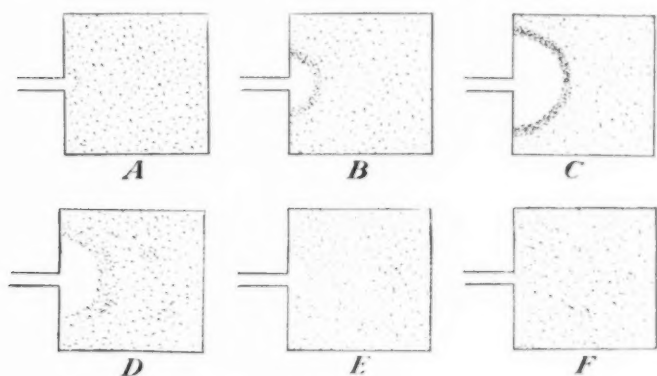


FIGURE 4.

surrounding the mouth of the diffusion tube (Fig. 4, *A*). A semi-circular area in which there are no organisms results. This area gradually becomes larger and is bounded by a ring in which the organisms are more densely gathered than they are in the surrounding culture medium (Fig. 4, *B*). This ring keeps pace with the enlarging clear area (Fig. 4, *C*). The enlargement continues, and if the acid is strong the ring is pushed to the periphery of the dish where the organisms scatter or die. If, however, the acid is not strong, the enlargement of the ring and clear area may stop sooner, in which case the ring slowly disappears (Fig. 4, *D*). The clear area, now un-

¹ In the experiments with *Chilomonas* much depends upon the conditions in the culture fluid. In order to equalize the conditions some of a dense culture of infusoria was diluted with a large amount of water before being submitted to an experiment. After this washing process constant results could be obtained. No experiment was recorded without experimental evidence that I could *not* obtain the same result with distilled water in the diffusion tube.

bounded by a ring, diminishes in size (Fig. 4, *E*), and finally recedes to the diffusion tube (Fig. 4, *F*), and no traces of the acid's action are apparent.

Are the formation of the ring and the clear area due to chemotropism or to chemokinesis? Observations showed that acids above a certain concentration cause the organism to become restless, very swift shooting movements being induced. The concentration of the acid varies inversely as the square of the distance from the mouth of the diffusion tube. At a certain distance from the mouth of the tube there is a line at which the acid becomes too weak to cause this comparative restlessness. Organisms moving from the area containing the strong acid and crossing this critical line at once

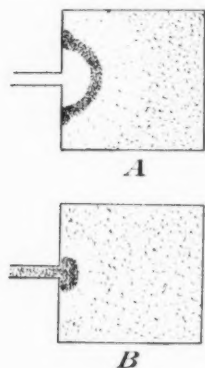


FIGURE 5.

become relatively quiet. The space just outside the critical line therefore acts like a trap in which the organisms are caught. In the course of time the critical line will move farther and farther from the mouth of the diffusion tube, necessarily causing the entrapped organisms to move with it. It is evident from these considerations that toward strong hydrochloric acid *Chilomonas* is chemokinetic. That, in the zone surrounding the clear area there is a dense gathering (in other words, that there is a ring formation), is in my opinion due to the fact that those individuals which were in the clear area are now gathered in the space immediately surrounding it. It might be argued that in

addition to this, another cause contributes to the formation of the ring, namely, that the weak acids might have an effect the reverse of that produced by strong acids. This would cause the infusoria to leave the periphery of the chamber and to gather nearer the critical line. Jennings indeed maintains that *Paramecia* are negative to strong acids and positive to weak acids, and thus explains the ring formation. But this is not the case for *Chilomonas*. I have never seen *Chilomonas* move toward an inorganic acid. If the acid is too weak to cause a clear area, no gathering ever takes place.

The fact that *Chilomonas* does not move toward an inorganic acid (*i. e.*, is neither chemokinetic nor positively chemotropic to inorganic acids) explains why the ring disappears and does not follow the clear

area in those experiments where the acid was too weak to cause the ring to move back to the periphery, and in which the clear area was seen to diminish in size after reaching a maximum (see page 205, Fig. 4, *D* and *E*).

The effects of lactic acid. — If lactic acid be substituted for hydrochloric acid the general behavior is somewhat different. A clear area bounded by a ring appears about the mouth of the diffusion tube just as in the case of hydrochloric acid, but the area does not increase in size as rapidly as when hydrochloric acid is employed. It is almost instantly surrounded by a ring in which the organisms are very densely gathered, much more densely than in the case of hydrochloric acid (Fig. 5, *A*). This ring does not disappear when the clear area has reached its maximum size, as in the case of hydrochloric acid; but when the clear area begins to recede again, the ring keeps pace with the change; and finally when the clear area has disappeared, the organisms of the ring form a dense plug in and about the mouth of the tube (Fig. 5, *B*).

How are these phenomena to be explained? I believe they are due to the fact that while for strong lactic acid the organisms are chemokinetic, for weaker lactic acid they are positively chemotropic.

A chemokinesis to strong lactic acid explains why a clear area is formed at the mouth of the tube and why the clear area increases in size. The second assumption, of a positive chemotropism to weaker lactic acid, explains why, after the acid has diffused until its concentration has fallen below a certain degree, the clear area and the ring become smaller and recede toward the mouth of the tube until they reach it.

That the organisms are positively chemotropic is decided by the following observation: —

Immediately outside the dense ring or gathering there is an area which shows a diminished number of organisms. It is easy to notice that the organisms within this area are oriented with the anterior ends (those bearing flagella) directed toward the ring, and that they are swimming in strictly radial lines, the lines of diffusion, toward the diffusing drop.

Hence those phenomena which indicate a *positive chemotropism* are produced by weak lactic acid.

II. THE EFFECTS OF IONS UPON THE AGGREGATION OF FLAGELLATES.

The use of chemically equivalent solutions.—In determining the effects of ions upon the movements of flagellates, Arrhenius's theory of electrolytic dissociation was necessarily used as a working basis. The older investigations of chemotropism, *e. g.*, those of Pfeffer,¹ did not deal with our problem for the simple reason that the dissociation of electrolytes was not yet known.

In making determinations of the chemotropic effects of ions equal percentage solutions cannot be compared. Yet even in recent literature such comparisons occur. For example, Jennings² compares the chemotropic effects of equal percentage solutions of various salts (LiCl, KCl, and others), and from the comparison draws the erroneous conclusion that lithium salts are "more repellent" than potassium salts, and that "the repellent power varies inversely with the atomic weight of the metal components." Jennings, to be sure, recognized the fact that a larger number of molecules of the lighter salt went into the solution, but did not see that this very fact contradicted the conclusions he had drawn. In addition, he did not consider the fact that in the solutions with which he worked the molecules were dissociated into ions; yet the ion concentration must be considered. If dissociation is complete, the effects produced by the solutions are the effects of all or of one class of the ions in the solutions. If the dissociation is not complete, the degree of dissociation must be determined.³

¹ PFEFFER: Untersuchungen aus dem botanischen Institut zu Tübingen, 1881-1885, i. p. 363.

² JENNINGS, H. S.: This journal, 1899, ii, p. 374.

³ To designate chemically equivalent solutions, the chemist uses fractional parts of the so-called "normal" solutions, *i. e.*, the solution made by dissolving the equivalent gram-molecule in one litre of water; *e. g.*, 36.5 grams of HCl per litre, or 3.65 per cent, is the normal solution; of H₂SO₄ (a dibasic acid), 98 grams per litre, or 4.9 per cent is the normal solution. If to 1 c.c. of a normal solution 99 c.c. of water be added, the dilution (V) = 100 and the fraction $\frac{N}{100}$ represents the strength of the dilute solution. If the dissociation is not complete, the degree of dissociation (a) can be computed from the electrical conductivity, $a = \frac{\mu_v}{\mu_\infty}$, in which equation μ_v represents the electrical conductivity at the dilution V (*i. e.*, $\frac{N}{V}$) and μ_∞ the conductivity when $V = \infty$, *i. e.*, when the dilution is infinite. The sum of the velocities of the ions for a given temperature gives μ_∞ .

The Alkalies.¹ — The specific chemical action of alkalies is due to the hydroxyl-ions. It is our intention to find out whether it is also the OH-ion which causes the reactions of infusoria subjected to the influence of alkalies.

The following alkalies were tested: LiOH, NaOH, KOH, Ca (OH)₂, Sr (OH)₂, Ba (OH)₂.

If these hydroxides act upon cultures of *Chilomonas* they invariably cause a clear area about the mouth of the tube when the solutions are as strong as $\frac{N}{500}$, and usually when they are considerably more dilute. At these dilutions the alkalies are completely dissociated into hydroxyl (OH-) ions (anions), and metal- or basic-ions (kations). The effect produced is therefore due solely to the action of ions; whether to the action of OH-ions or kations is decided by the following fact: $\frac{N}{500}$ solutions of the corresponding chlorides, and even solutions of three times this strength, produce no apparent effect upon *Chilomonas*. Such solutions of the chlorides are completely dissociated. It follows then that the formation of the clear area about the mouth of the tube by the action of alkalies as dilute as $\frac{N}{500}$ is due to the action of the *hydroxyl-ions*. This deduction is further supported by the fact that no difference can be distinguished in the phenomena presented by *Chilomonas* cultures subjected to the actions of chemically equivalent solutions of the different alkalies. Barium hydroxide seems to act in precisely the same manner and with the same intensity as sodium hydroxide, provided the concentration of OH-ions is the same in both cases.

It has already been stated that $\frac{N}{500}$ and stronger solutions of the alkalies invariably cause the clear area about the mouth of the tube. $\frac{N}{1000}$ solutions are strong enough to do this in most cases, while at times the organism is sensitive to solutions as dilute as $\frac{N}{2000}$. This variation is due to the different sensitiveness of the infusoria of different cultures and to the fact that the sensitiveness of the organism in any culture varies from day to day. If the same culture be employed in all the experiments of a single day, the results are constant.

If a strong solution of alkali is tested, a ring is formed similar to that formed about diffusing hydrochloric acid and already described. In this connection no orientation of the infusoria can be detected. The ring is only temporary and cannot be produced by dilute solutions. From these facts I conclude that *Chilomonas* is chemokinetic to OH-ions.

¹ Since strong alkalies attack hard rubber the experiments were repeated in an apparatus made entirely of glass.

Solutions of the carbonates of those bases whose hydroxides were tested contain free hydroxyl-ions. These carbonates in our experiments acted like the hydroxides. The conditions in such solutions are too complex to allow the establishment of any quantitative relations; I can only state that the typical clear area appeared with solutions of Na_2CO_3 , K_2CO_3 , and other carbonates so dilute that chemically equivalent solutions of the chlorides of corresponding metals produced no effect. The effects produced by the carbonates then are not due to the kations, but are due either to the hydroxyl-ions or to CO_3 -ions or to the undissociated molecules.

The behavior of *Chilomonas* toward NH_4OH was also tested. The experiments yielded precisely the same quantitative results as did those with the other alkalis. Due to incomplete dissociation, however, the number of OH -ions is much smaller in solutions of NH_4OH than in chemically equivalent solutions of the other alkalis. The effect, then, cannot be due to the OH -ions alone, but there is probably added to it the effect of the NH_4 -ions or the undissociated NH_4OH molecules, or both.

Inorganic acids.—The common properties of acid solutions are due to the hydrogen-ions present. This statement is true for general chemical reactions, as well as for the effects obtained when *Chilomonas* cultures are subjected to the action of chemically equivalent solutions of HCl , HNO_3 , or H_2SO_4 . The phenomena observable have already been described in the general discussion of the reactions of the organism to HCl . It remains to consider the action of *ions* in producing the phenomena.

The characteristic clear area appears about the mouth of the diffusion tube whenever solutions of these inorganic acids as strong as $\frac{1}{1000}$ are tested. At the dilution $\frac{1}{1000}$ each of these acids is completely dissociated into H -ions and the anions characteristic of the acid.

Is the effect due to the H -ions or to the anions? To decide this, experiments were made with salts that contained the same anion as the acid. It was found that a chemically equivalent solution of the sodium salt of any of these acids, or even a solution ten times as strong, had no effect upon *Chilomonas*. Since in these solutions the salt is completely dissociated and the anions produce no effect, the effects produced by the acids can be due only to the H -ions.

As was noted in considering the effects of the hydroxyl-ion, *Chilomonas* does not always exhibit the same sensitiveness in its reaction

toward the same ions. The hydrogen-ion invariably causes the clear space when the solution of the inorganic acid is $\frac{1}{15000}$ and sometimes when as dilute as $\frac{1}{30000}$. If the solution in the tube was $\frac{1}{40000}$ or $\frac{1}{50000}$ the organism showed a sensitiveness only at the instant of contact with it. The most common dilution of the inorganic acids beyond which the clear area was not formed, was about $\frac{1}{12000}$ to $\frac{1}{18000}$.

If a comparison be made between the action of the H-ion and OH-ion, it is evident that the H-ion is the more active. The ratio of activity is about 2:1. If a solution of mineral acid be so diluted that the clear area is barely evident, it will require a solution of alkali almost twice as strong to produce the same effect. This fact is significant when considered in the light of Loeb's observation that within the various groups of the natural system the poisonous effects of ions upon muscle are, to a certain extent, a function of their velocity.¹ The ratio of the velocity of the H-ion to that of the OH-ion is, according to Ostwald, 325 to 170, when the temperature is 25° C. This ratio is nearly 2:1, or the same as their effect upon *Chilomonas*. Jennings states that in his experiments with *Paramecia* "the results with acids (inorganic) are not due to the hydrogen-ion of the acids," but to the anions.² The acids used by Jennings were completely dissociated, and the hydrogen-ions are the only ones common to all the acids. The anions cannot produce the effects as will be further shown in the discussion of the effects of inorganic salts, and we are forced to the conclusion that *it is the H-ion of inorganic acids* which produces the observed effects.

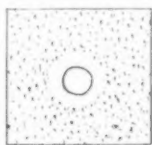


FIGURE 6.

Solutions of carbon dioxide have acid reactions due to the fact that free hydrogen-ions are formed by the electrolytic dissociation of carbonic acid (H_2CO_3). It would be expected, *a priori*, that the behavior of *Chilomonas* toward a bubble of CO_2 or, more exactly, toward the carbonic acid solution surrounding the bubble, would be similar to that toward *weak* solutions of other inorganic acids. This is, in fact, the case. A clear area forms about the bubble at a short distance from it, as is shown diagrammatically in Fig. 6. The organism does not at any time swim to the bubble as if attracted by it. No

¹ LOEB, J.: *Archiv f. d. ges. Physiol.*, 1897, lxi, p. 21.

² JENNINGS, H. S.: *This journal*, 1899, ii, p. 370.

ring or gathering of any kind is noticeable.¹ Aqueous solutions of carbon dioxide introduced into the diffusion tube had the same effect upon *Chilomonas* as the bubble of CO_2 or as *weak* solutions of the other inorganic acids. This effect of H_2CO_3 in a solution of CO_2 is probably due largely to H-ions, but since the degree of electrolytic dissociation of H_2CO_3 is not high, the molecules of the acid may play a large rôle. Another factor may be CO_2 molecules in simple solution.

Salts of inorganic acids.—The relative action of the ions of the inorganic salts was determined by testing groups of these salts in series, *e. g.*, to find the relative action of the Li-, Na-, and K-ions, series of chlorides of these bases were tested. Chemically equivalent solutions of these salts have the same number of chlorine ions, and differences in the reactions of *Chilomonas* to these solutions are due to differences in the specific activities of the kations. The relative effects of the kations can be determined by comparing various salts of inorganic acids. Similarly, the effects of the anions may be determined by comparing the Li-salts or Na-salts or K-salts of the various acids. In trying to determine the effects of anions it is expedient to select salts in which the kation is comparatively ineffective, and *vice versa*.

To determine the action of the anions Cl, Br, and I, series of the lithium salts, sodium salts, and potassium salts were separately tested. In every case no effect was produced upon *Chilomonas* cultures except the formation of the clear area about the mouth of the diffusion tube in which the salts were placed. If $\frac{1}{10}$ NaCl was gradually diluted until the solution no longer produced the clear area, it was found that

¹ These investigations with *Chilomonas* agree with the investigations of Loeb and Hardesty,¹ who found that *Paramecium* left those regions where CO_2 was entering into solution. Jennings claimed to have found *Paramecium* positively "attracted" to CO_2 .²

I made series of experiments with *Paramecia* (taken directly from the culture, and also washed with distilled water) with the intention of confirming Jennings's statement. I introduced CO_2 bubbles under a cover-slip; in another experiment the gas was led through the Engelmann chamber in which *Paramecia* were observed; while in a third series I tried the effects of water saturated with H_2CO_3 —but always with a result contrary to that of Jennings. The *Paramecia* were not "attracted" to CO_2 . Carbon dioxide acts upon *Paramecium* as it does upon *Chilomonas*, *i. e.*, just as all inorganic acids do.

¹ LOEB, J., and L. HARDESTY: *Archiv f. d. ges. Physiol.*, 1895, lxi, p. 591.

² JENNINGS, H. S.: *Journal of physiology*, 1897, xxi, p. 288 et seq.

chemically equivalent solutions of NaBr and NaI were still strong enough to produce it. At a dilution at which the NaBr ceased to have any perceptible effect upon the organism of the culture, NaI still caused the formation of the clear area. The lithium and potassium series gave results similar to those obtained with the sodium series. The table below gives the results of some typical experiments. The strengths given represent the dilution at which the clear area was no longer formed.

TABLE I.

	Cl.	Br.*	I.
Li	N 17	N 43	N 56
Na	N 20	N 39	N 55
K	N 45	N 50	N 80

At the dilutions given in the table, the salts may be considered completely dissociated, and any effect will be due to the action of the ions. In the less dilute solutions convection currents were noticeable and it was thought that the formation of the clear area might have been due to some physical action of the solution dependent upon the specific gravity. The comparison of the strengths at which perceptible effects ceased shows that the action of the solutions is not proportional to the specific gravities. To test the question further the action of solutions of glucose toward the organism was examined. Solutions of glucose with a specific gravity greater than that of any of the solutions of salts mentioned in Table I did not cause the formation of the clear area about the mouth of the diffusion tube. Convection currents were noticeable, but no direct effect upon the organism, which was able to swim, though slowly, into the solutions. The conclusion that the effect of the solutions of salts considered above upon *Chilomonas* is a chemical one is justifiable.

The difference of chemically equivalent solutions of the halogen salts of Na, or of Li, or of K is due to the different effects of the ions Cl, Br, and I. Of these ions, Cl is the least active, Br next, and I the most active. The ratio of activity seems to be about Cl:Br:I::2:3:5. The ions are monatomic, and their effect upon *Chilomonas* increases with their atomic weight, though it is not necessarily a

function of their atomic weight. Kahlenberg¹ found that the salty taste of the halogen ions *decreases* as the atomic weight increases.

The effect of chemically equivalent solutions of LiCl and NaCl upon *Chilomonas* cultures is about equal. Solutions of KCl will cause the characteristic clear area about the mouth of the diffusion tube at dilutions at which solutions of NaCl or LiCl have no effect. When $\frac{N}{20}$, LiCl, or NaCl no longer have this effect, $\frac{N}{27}$ to $\frac{N}{30}$ KCl is still just able to produce the clear area, a fact which agrees with Loeb's experience.

Comparison of the bromides gives analogous results. KBr causes the formation of the clear area in solutions as dilute as $\frac{N}{10}$ to $\frac{N}{50}$, while solutions of LiBr and NaBr must be as strong as $\frac{N}{30}$ to produce the same effect. These solutions are completely dissociated.

From these facts we draw the conclusion that, so far as *Chilomonas* cultures are concerned, the Li- and Na-ions have equal effect and that the K-ions are more active than the other two.

In order to give the reader an idea of how much the individual experiments vary I add Table II, which gives the result obtained in a series of six experiments with the solutions just considered. This result is typical of a large series. The strengths of the solutions given in the table denote the dilution necessary to just prevent the formation of a clear area when the solution is placed in the diffusion tube.

TABLE II.

	1	2	3	4	5	6
LiCl	$\frac{N}{15}$	$\frac{N}{50}$	$\frac{N}{17}$	$\frac{N}{30}$	$\frac{N}{30}$
NaCl	$\frac{N}{20}$	$\frac{N}{30}$	$\frac{N}{20}$
KCl	$\frac{N}{27}$ $\frac{N}{30}$	$\frac{N}{70}$	$\frac{N}{32}$	$\frac{N}{60}$	$\frac{N}{35}$	$\frac{N}{80}$
LiBr	$\frac{N}{30}$	$\frac{N}{80}$	$\frac{N}{33}$	$\frac{N}{80}$
NaBr	$\frac{N}{30}$	$\frac{N}{73}$	$\frac{N}{32}$
KBr	$\frac{N}{30-40}$	$\frac{N}{110}$	$\frac{N}{50}$	$\frac{N}{50}$
LiI	$\frac{N}{50}$
KI	$\frac{N}{80}$

¹ KAHLBERG, L.: Bulletin of the University of Wisconsin, Madison, Sept., 1898, No. 25.

No difference could be detected in the reaction of *Chilomonas* to equimolecular solutions of calcium chloride and strontium chloride. Magnesium chloride was less active than either, producing the same effect only by stronger solutions. Barium chloride was the most active of the chlorides of the alkali earths. If the solutions of the other members of this series were diluted until they just failed to produce an effect on *Chilomonas*, an equivalent solution of barium chloride was markedly active in the formation of the clear area about the mouth of the diffusion tube. Three series of experiments are given in the subjoined table, showing the dilutions at which solutions of the salts cease to produce the formation of the clear area in a culture of *Chilomonas*.

TABLE III

	1	2	3
MgCl ₂	N 7.5	N 10.0	N 6.0
CaCl ₂	N 10.8	N 13.5	N 9.5
SrCl ₂	N 11.6	N 14.0	N 10.0
BaCl ₂	N 17.5	N 21.5	N 15.0

A clear area appears in the *Chilomonas* culture about the mouth of the diffusion tube whenever the solutions of the salts are a trifle stronger than the figures in the table indicate. At such strengths the salts may be considered completely dissociated and the effects are those of ions. Differences in the action of these salts can only be due to the different metal constituents. We must not, however, overlook the fact that another kation may co-operate with it. These salts are salts of bivalent metals and the molecule may dissociate in two ways: *e. g.* MgCl₂ = Mg⁺, Cl⁻, Cl⁻, or = MgCl⁺, Cl⁻. I cannot state what share each of the two possible kations has in the action of each of the solutions.

The ratio of the effects of chlorides of Mg: Ca: Sr: Ba is 3: 5: 5: 7, nearly.

Comparison between the reaction of *Chilomonas* to solutions of KCl and $\frac{1}{2}$ CaCl₂ showed that calcium chloride in $\frac{1}{2}$ solutions was as effective as $\frac{1}{2}$ solutions of potassium chloride. Ignoring the possibility of the presence and action of the compound kation CaCl,

we may make the statement that the ion Ca has a more marked influence on the reactions of *Chilomonas* than two ions of K.

The reaction of *Chilomonas* to solutions of the following salts of the heavy metals was observed: FeCl_2 , ZnSO_4 , ZnCl_2 , CuSO_4 , CuCl_2 , CuCl , HgCl_2 , and AgNO_3 . No effort was made to determine *accurately* the dilution at which reactions to these salts ceased. It was found, however, that these salts in very dilute solutions produced the typical clear area, and often the ring. In most cases the effect followed when the solutions were more dilute than 1000^{N} to 2000^{N} . It has already been shown that anions of these salts cannot produce any effect upon *Chilomonas* when the solutions are as dilute as this. Since the salts are largely dissociated, we conclude that the heavy-metal kations are the active factors which caused the reaction of *Chilomonas*.

The conditions in some solutions of the heavy metals (*e. g.* CuSO_4) are slightly complicated by the fact that these solutions contain a small number of free H-ions. The effects produced by these solutions simulate those produced by the inorganic acids. Are the free H-ions responsible for the effects? I think not. Suppose that the solution contained as many H-ions as 10 per cent of the number of salt molecules (the number is actually much less than 10 per cent); the acidity of a 1000^{N} solution of one of these salts would then be equivalent to that of a 10000^{N} solution of acid. The characteristic reaction can be obtained with all the above salts in 1000^{N} solutions, but no 10000^{N} acid could produce these effects, and we are forced to conclude that *the effects of such solutions are not, or at least not exclusively, the effects of the free H-ions.*

Jennings concluded from his work on *Paramecia* that "salts that contain the relatively inactive kation of one of the heavy metals as aluminium, copper, zinc, mercury, produce an effect due only to the anion, hence the effect is like that of an acid."¹ My experiments upon *Chilomonas* show that it is the H-ion, not the anions, that produce the effects of inorganic acids; that the *anions* are relatively inactive; and that *the heavy-metal kations are extremely active.*

Organic acids. — In dealing with the reaction of *Chilomonas* toward inorganic acids, we showed that the conditions existing in the solutions were comparatively simple. Solutions not strong enough to kill the organism outright were completely dissociated and their effects were due to ions, — in fact, when dilute, could be ascribed to H-ions only. With solutions of organic acids, the conditions are

¹ JENNINGS, H. S.: This journal, 1899, ii, p. 370.

more complex. Dissociation is not complete. Besides the H-ions and complex anions, undissociated molecules are present and undoubtedly play no inconsiderable rôle in the various phenomena seen when the solutions act upon cultures of *Chilomonas*.

Solutions of the following organic acids were experimented with:

I.	II.	III.
Oxalic.	Malic.	Acetic.
Formic.	Tartaric (Dextral).	Lactic.
Citric.	Mandelic.	Butyric.
Succinic.		
Valerianic.		

According to their effect upon the reactions of *Chilomonas*, these acids may be divided for convenience into three groups:—

Group I. The first five acids of the above list comprise the first group. Like the inorganic acids they cause the clear area and, when strong, a temporary and never dense ring about the area. The ring was not formed by a migration of the organism from without to it. No other effects were observable. *Chilomonas* is chemokinetic to these acids.

Group II. Malic, tartaric, and mandelic acids produce a clear area, often with a ring about it. In the formation of the ring, the phenomena were so inconstant that I was unable to say that it was or was not due to a migration of the organisms from without to it. To these acids *Chilomonas* is chemokinetic; whether in addition it is positively chemotropic I cannot say.

Group III. Acetic, lactic, and butyric acids acting upon *Chilomonas* present the phenomena already described in the first part of this paper: a clear area surrounded by a dense ring (see Fig. 5). *Chilomonas* is chemokinetic towards strong solutions of these acids, and is positively chemotropic toward weaker solutions.

The acids of all these groups when sufficiently strong produced the characteristic clear area about the mouth of the diffusion tube noted in all experiments upon *Chilomonas* which have thus far been considered. Inorganic acids as dilute as $\frac{N}{1000}$ or $\frac{N}{1200}$ invariably caused this characteristic clear area, and we concluded that the effects were due to the action of H-ions only. Is the similar effect of organic acids due to the H-ions? If so, we would expect the organic acids to produce the clear area when their solutions were of such strengths that they contained the same number of H-ions as $\frac{N}{1000}$ or $\frac{N}{1200}$ solutions of the inorganic acids.

Oxalic acid never produces the clear area when more dilute than $\frac{N}{700}$. In many cases only solutions stronger than $\frac{N}{300}$ have this effect upon *Chilomonas*. At the strength $\frac{N}{300}$ oxalic acid is largely dissociated and therefore contains about three times as many H-ions as $\frac{N}{1000}$ solutions of inorganic acids like HCl which produce the same effect. The action of the oxalic acid is thus only one third as strong as it should be according to the number of H-ions.

Formic acid is also only about one third as active as is to be expected from its content of H-ions. It requires a solution as strong as $\frac{N}{100}$ to invariably produce the clear area. In $\frac{N}{100}$ formic acid about 33 per cent of the molecules are dissociated. It contains about the same number of H-ions as a $\frac{N}{300}$ solution of an inorganic acid, *i. e.*, more than three times as many as a $\frac{N}{1000}$ solution of an inorganic acid which produces an equivalent effect. In $\frac{N}{300}$ tartaric (dextro) acid about 25 per cent of the molecules are dissociated, and so far as H-ions are concerned, it equals $\frac{N}{1200}$ solutions of inorganic acids like HCl, and produces about the same effects as they do.

On account of the dense ring about the clear area it is not easy to determine with certainty that degree of dilution of acetic acid which no longer causes the formation of the clear area. The following figures are selected to show the variations on different days:

TABLE IV.

1	2	3	4	5	6	7	8
$\frac{N}{300}$	$\frac{N}{300}$	$\frac{N}{200-400}$	$\frac{N}{300-400}$	$\frac{N}{400}$	$\frac{N}{300}$	$\frac{N}{200-400}$	$\frac{N}{300-500}$

It is seen from these data that the clear area is usually formed as a result of the action of acetic acid on *Chilomonas* cultures when the solution is as dilute as $\frac{N}{300}$, and it is invariably formed when the solution is as strong as $\frac{N}{200}$. In $\frac{N}{200}$ solutions of acetic acid about 6.2 per cent (18° C.) of the molecules are dissociated, whereas in equimolecular hydrochloric acid about 95 per cent are dissociated. The HCl solutions therefore contain 15.3 times as many H-ions as the acetic acid. If the HCl be diluted to $\frac{N}{1000}$, it will produce the same effect as $\frac{N}{200}$ acetic acid. But even such a solution will contain more than three times as many hydrogen-ions as $\frac{N}{200}$ acetic acid. The action of acetic acid in the formation of the clear area in cultures of *Chilomonas* is therefore more than three times as great as it should be if the H-ions were the *oxy* factor in the reaction.

In the formation of the clear area, lactic acid is about as active as HCl, the clear area being always formed by 1_{10000} solutions. At this strength and at a temperature of 19° , only 29 per cent of the lactic acid molecules are dissociated, and this acid therefore contains less than one third as many H-ions as the HCl with which it is equally active.

We therefore see that there is a great difference in the effects of organic and inorganic acids upon the reactions of *Chilomonas*. While inorganic acids show the same effects if they contain the same number of H-ions in the unit volume of the solution, the *organic acids do not show a definite relation between the number of H-ions and the effects produced*. This fact corresponds with what Loeb found previously concerning the effects of acids upon muscle. He showed that the addition of small amounts of acid to physiological salt solutions causes the muscle to take up a considerable amount of water. In the case of the inorganic acids HCl, HNO₃, H₂SO₄, KHSO₄, NaHSO₄ the amount of water taken up in a given time by the same mass of muscle is the same, if the solutions contain the same number of H-ions in the unit volume. But in the effects of organic acids such simple conditions do not exist. Acetic, and still more so, lactic acid acts much more strongly than is to be expected from the degree of dissociation (*i. e.* from the number of free H-ions in the solution).

Loeb¹ suggests that these differences in the behavior of organic and inorganic acids may be due to the fact that those organic acids which are very incompletely dissociated (*e. g.*, acetic and lactic acids) are transformed inside the muscle into products which undergo a greater degree of dissociation.

Richards² and Kahlenberg³ in their investigations on taste, found similar variations in the effects of organic acids. Both attribute the sour taste of acid to the H-ions in the solution. Both, however, found that by comparison with inorganic acids the sour taste of acetic acid was about three times as intense as the number of H-ions warranted. No explanation of these phenomena is offered by them.

Later, in a review of Professor Loeb's work on effects of ions, Ostwald⁴ makes the following suggestion concerning the variations ob-

¹ LOEB, J.: Archiv f. d. ges. Physiol., 1898, lxxi, p. 460.

² RICHARDS, TH.: American chemical journal, 1898, xx, p. 121.

³ KAHLENBERG, L.: Bulletin of the University of Wisconsin, 1898, No. 25.

⁴ OSTWALD: Zeitschrift für physikalische Chemie, 1899, xxviii, p. 174.

served when organic acids are used: "The phenomena observed here and with taste sensations would be explained if it were assumed that the cells contain the neutral salts of an acid of medium strength, the anion of which reacts with the hydrogen-ion." Similar transformations may explain the behavior of the organisms in our experiments on the reactions of *Chilomonas* to chemicals.

It has already been pointed out that *Chilomonas* cultures vary in their sensitiveness from day to day, and it was noticed that on those days when the organism was most sensitive toward H-ions, *i. e.*, when the clear area was formed by the action of very dilute inorganic acids, it showed a proportional sensitiveness toward organic acids. The sensitiveness toward ions other than H, *e. g.* Na, or Cl, did not necessarily vary on those days.

Positive chemotropism to organic acids.—We have already mentioned the fact that lactic acid, besides producing the clear area, has the property of causing a positively chemotropic *orientation* of *Chilomonas*. This orientation is also a characteristic effect of acetic and butyric acids and is responsible for dense gatherings of *Chilomonas* subjected to their influence. If the solution of acid is strong, the gathering has the form of a very dense ring surrounding a clear area. If the solution is sufficiently dilute, the clear area is not formed but a dense gathering into the acid in and about the mouth of the diffusion tube results. The organism is oriented just as when put under the influence of a galvanic current,¹ and migrates along radially disposed lines toward the centre of the diffusing drop. This migration was most markedly evident in two or three experiments in which the organisms were gathered very densely about debris situated some distance from the mouth of the diffusion tube. As soon as they came under the influence of the diffusing acid the organisms left the debris and fairly swarmed into the acid. *The gathering toward these organic acids is due to a positive chemotropism, to an orientation in the lines of diffusion, and a definite movement into the acids.*

On account of the minuteness of *Chilomonas* and the consequent difficulty in observing its flagella, a study of the mechanics by which the organism is oriented, or by which it is prevented from moving from the ring into the stronger acid of the clear area, or the weaker acid surrounding the ring, proved fruitless.

The ring obtained with the inorganic acids is only temporary if the acid is not too strong. In the course of a short time all traces of the

¹ I found that the organism moves towards the anode.

acid's action disappear. On the other hand, the ring obtained with acetic, lactic, or butyric acids is more permanent. In the latter case the gathering has been observed under most favorable conditions to persist as long as three hours, at the end of which time the evidence of its influence disappeared as the diffusion caused the acid to become distributed evenly in the chamber. Those rings which persist some time were observed to split along their entire length, each ring giving rise to two concentric rings (Fig. 7), the inner of which dissolved, leaving only the outer, which retained very nearly its original position and repeated the splitting process. Three such consecutive splittings have been observed before the ring disappeared. Exactly similar phenomena of splitting were observed in the rings which formed about bubbles of air and which remained intact long enough (six hours). In very dense collections, *i. g.*, about debris, the organisms were also seen to move away from the centre of the dense gathering, leaving a small clear area there.

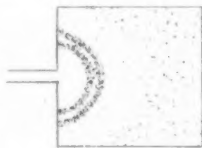


FIG. 7.

Solutions of acetic, lactic, and butyric acids as dilute as $\frac{1}{100,000}$ to $\frac{1}{500,000}$ have been observed to cause dense gatherings of *Chilomonas*; *i. e.*, to produce positive chemotropism. Such gatherings are not, however, usually formed by the action of solutions more dilute than $\frac{1}{300,000}$.

The point of interest is: Why do acetic, lactic, and butyric acids produce positive chemotropism if they are sufficiently weak, while the other acids produce no such effect? In solutions of these acids which are even as dilute as $\frac{1}{300,000}$, less than one fifth of the molecules are dissociated. There are then three possible factors; H-ions, anions, undissociated molecules.

It has already been shown that the H-ion does not cause any positive chemotropism of *Chilomonas*. We have then to decide between the undissociated molecule and the anion as the active agent, though it is not impossible that both may be factors. It is possible to obtain *other* compounds (salts) which do not contain the acid molecules but do *contain the anions* that are contained in the acid solutions. If the anion of lactic, of butyric, or of acetic acids be the cause of the positive chemotropism reaction of *Chilomonas*, it is to be expected that the salts of these three acids will cause the positive chemotropism in the same way that the acids do.

Salts of organic acids.—Actual experiments showed that sodium oxalate, ammonium oxalate, sodium citrate, and sodium tartrate do not cause any positive chemotropism of *Chilomonas*. They act qualitatively like the salts of the inorganic acid. The salts mentioned are salts of those inorganic acids which I placed in Groups I and II, acids which do not cause any positive chemotropism of *Chilomonas*, or concerning the action of which I am uncertain. *The sodium salts of acetic and butyric acids do, however, cause the positive chemotropic reaction of Chilomonas.* When acted upon by solutions of these salts the organisms swim toward the centre of diffusion along radial lines. The migration soon leads to the formation of a ring outside of which there exists a broad area where there are only a few infusoria, all of which are oriented and swimming

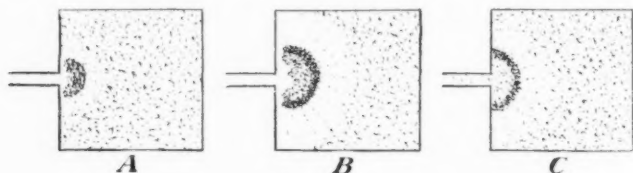


FIGURE 8.

toward the ring. The ring is always densest along its outer border, probably because it is continually receiving additions from the outside and because the ring continually broadens toward the inside. The inner border of the ring is not always sharp and regular as is the case when acids are used. The organisms can move from the densest part of the ring and up into the diffusion tube when the solution within it is as strong as $\frac{N}{8}$. Usually the gathering appears as a patch, which presents a granular aspect due to the uneven distribution of the organisms (Fig. 8, A). Very soon this patch is framed by a dense ring (Fig. 8, B). If the solutions used are dilute, the gathering has the appearance shown in Fig. 8, C.

Chilomonas was positively chemotropic toward solutions of sodium acetate and sodium butyrate which were as dilute as $\frac{N}{4000}$, and in some cases still showed the effects when the solutions were as dilute as $\frac{N}{8000}$. If the anion CH_3COO is the cause of the positive chemotropism of *Chilomonas* to acetic acid or sodium acetate, it is to be expected that the organisms will also be positively chemotropic to other acetates. Solutions of the acetates of copper, iron, and ammonium were tested. *Chilomonas* was positively chemotropic toward

all three. In the case of ammonium acetate, acetic acid is present, and the orientation may be due to its action. No quantitative work was done with these salts.

From these facts we conclude that in the case of lactic, acetic, and butyric acids we have two effects upon *Chilomonas*:

1. Chemokinesis produced by the H-ions.
2. *Positive chemotropism* produced by the anions (or by the undissociated molecules).

Pfeffer found that the spermatozooids of ferns were positively chemotropic both to malic acid and its salts. My experiments make it seem highly probable that the effects are due to the anion in the solutions and not to the acid (H-ions) as has thus far been commonly accepted.

Jennings recently expressed the view that "in and of itself there is no positive taxis (tropism) of *Paramecium*" but that all gatherings are brought about by a certain "motor reaction by which it responds to all classes of stimuli" and that the terms "positive and negative taxis (tropism) as applied to *Paramecium* are merely convenient terms for expressing the fact that the animals form collections"¹ or do not. *Orientation and tropism are synonymous expressions*; that *gatherings* take place is a *consequence of the orientation*, and is purely incidental. Jennings's "motor reaction"² cannot account for an orientation, and therefore throws no light whatever upon the tropisms. (Gatherings, however, are not always indicative of a tropism, *i. e.*, of an orientation, but may be due to kinesis (*Unterschiedsempfindlichkeit*), as Loeb showed many years ago, a matter that has already received sufficient notice in the preceding pages of this article).

¹ JENNINGS: This journal, 1899, ii, pp. 311 *et seq.*

² Jennings will pardon me if I call his attention to the fact that the principle of a motor reaction is nothing new. If we apply a sudden local stimulus to a muscle it always reacts with the same motion: it twitches, no matter where we apply the stimulus, and no matter whether the stimulus be electrical, mechanical, or chemical. Likewise, a frog shows the same motor reaction if we suddenly give it an electric shock, or strike it, or touch it with acetic acid: it jumps, as everybody knows, and this same motor reaction occurs, whether the stimulus be applied to the head, to the fore legs, or to the hind legs. The sensitive plant shows a motor reaction: even a human being may show a motor reaction if caught unawares. But all these motor reactions have nothing to do with the *tropisms*, for these motor reactions are only the expression of a very sudden change of the stimuli, while the characteristic of the tropism is the stationary condition of the stimuli.

SUMMARY.

1. There exists a complete analogy between the effects of chemicals upon the reactions of the flagellated infusorian *Chilomonas*, and the effects of other stimuli, *e. g.*, light or the galvanic current, upon certain other forms. We are able to distinguish two causes for the formation of gatherings: first, chemokinesis (*Unterschiedsempfindlichkeit*) and second, positive chemotropism.

2. In determining the effect of ions upon the reactions of organisms, equal percentage solutions must not be compared, but only solutions which have the same ion-concentration.

3. Toward the alkalies LiOH , NaOH , KOH , CaOH_2 , SrOH_2 , BaOH_2 , *Chilomonas* shows chemokinesis and the effect is due to the OH -ions. Chemically equivalent solutions of these alkalies contain the same number of OH -ions per unit volume, and therefore produce equal effects.

4. Toward the inorganic acids, HCl , HNO_3 , H_2SO_4 , *Chilomonas* shows chemokinesis, and the effects are due to the H -ions. If the solutions of these acids have the same concentration of H -ions, they have equal effects. H -ions are more powerful than OH -ions in causing these reactions of *Chilomonas* and apparently in the ratio of the ion velocities.

5. The effect which the carbonates of the so-called alkali-metals and alkali-earths produce upon the reactions of *Chilomonas*, is qualitatively similar to that of the alkalies, and is probably due to the OH -ions, though it may be partly due to CO_3 -ions, or undissociated molecules.

6. Upon the reactions of *Chilomonas* NH_4OH , though very incompletely dissociated, has as marked effects as the other alkalies. To the effects of the OH -ions is added that of some other factors, — NH_4 -ions or NH_4OH -molecules.

7. The effects of carbon dioxide upon *Chilomonas* are really those of carbonic acid (H_2CO_3) and are the same as the effects of other weak inorganic acids.

8. A series of inorganic salts of Fe , Zn , Cu , Hg , and Ag showed that their effect upon the reactions of *Chilomonas* was chemokinetic and due to the kations. Strong solutions of many of these salts contain free H -ions. The effects can, however, be obtained at dilutions which preclude the possibility of an exclusive effect of the H -ions.

9. Toward stimulation by *organic* acids *Chilomonas* shows as a rule chemokinesis. While inorganic acids have equal effects if the ion-concentration is the same, organic acids behave differently. In some cases, *e. g.*, that of acetic acid, the effects are greater than could be expected from the degree of dissociation. This result is similar to that previously found by Loeb on the effects of acids upon the absorption of liquids by muscle, and by Richards and Kahlenberg in their experiments on the taste of acids.

It is possible, as Loeb and Ostwald suggest, that within the tissue the organic acids are transformed into other compounds; the products of these transformations might account for the differences in the effects of organic and inorganic acids.

10. If we use very dilute solutions of certain organic acids, for example dilute solutions of acetic, lactic, and butyric acids, we find that they produce altogether different effects than those produced by solutions of any of the other compounds thus far considered. Toward these solutions *Chilomonas* is positively chemotropic. These positively chemotropic effects are due to the action of the anions or to the undissociated molecules. I was able to show that salts of acetic acid produced positively chemotropic effects when the dilution corresponded to that dilution of acetic acid which produced the positively chemotropic effects. This supports the conclusion that the effect is due to the anion.

I desire to thank Professor Loeb, not only for pointing out the possibilities in this line of investigation and for valuable suggestions as to methods of experimentation, but also for his kindly encouragement, which has made my task a most pleasant one.

ON THE CHEMICAL REACTION OF THE INTESTINAL
CONTENTS TO VARIOUS INDICATORS, AND ON THE
NATURE OF THE CONTENTS ESCAPING FROM A
FISTULA IMMEDIATELY ABOVE THE ILEO-CÆCAL
VALVE.

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IT might be supposed that a point apparently so easy to determine as the reaction of the intestinal contents would long ago have been definitely settled. Such, however, is not the case, for after a long period during which it was constantly taught with confidence by the text-books that the intestine had an alkaline reaction, the view has recently been advanced that the reaction is an acid one, due chiefly to organic acids, the product of bacterial decomposition of carbohydrates, and this view has found its way into several recent text-books. More recently still, it has been stated that the total acidity of the contents of the duodenum is almost always above that of the gastric contents, while the acidity present in the large intestine is usually as great as, and often greater than, that of the duodenal contents.¹ It has also been stated that the acidity of the intestinal contents is in part due to hydrochloric acid combined with proteid, and not entirely to organic acids or acid salts.²

According to other recent observations, the intestinal contents are almost invariably alkaline from the pylorus, or within a few centimetres of it, onwards; the contents are also said to be more alkaline in herbivora, where the diet contains more carbohydrate than in carnivora; and further the reaction is said to become much more strongly alkaline in carnivora under carbohydrate feeding and to approximate to the stronger alkaline reaction of the herbivora.³ Such increased alkalinity under carbohydrate feeding is opposed to the view that under normal conditions there is any appreciable decomposition of

¹ GILLESPIE: Proceedings of the Royal Society, London, 1897, lxii, p. 5.

² GILLESPIE: *loc. cit.*, p. 10.

³ MOORE and ROCKWOOD: Journal of physiology, 1897, xxi, p. 380. In this paper the literature of the subject is given to date, and a historic discussion in the text has accordingly been omitted.

carbohydrate by bacterial agency in the small intestine accompanied by the setting free of organic acids such as acetic and lactic. There is then, it may be observed, wide divergency of opinion, even as to the simple question of reaction and its causation, some finding an acidity due to acetic and lactic acids, others an additional acidity due to hydrochloric acid, while others again state that the alkaline reaction precludes the possibility either of hydrochloric acid combined with proteid or of any strong organic acid such as lactic or acetic.

These conflicting statements clearly demonstrate that the observation of the reaction is not so easy as it at first sight appears. The reason is in part that the alkalinity or acidity, whichever be found, is never due to free acid or free alkali, but to combined acid or combined alkali which do not give identical reactions with different indicators, appearing acid to some and alkaline to others. These different indicators have been used somewhat indiscriminately by the different observers and the variation in the results obtained is partly due to this cause and partly to the fact that the reaction has in some cases been determined under pathological conditions, especially in case of the reaction of the human intestine.¹

The experimental fact that the intestinal contents give a varying reaction with different indicators, seems at first sight to increase the complexity of the problem and to render it impossible to determine with definiteness whether the reaction is acid or alkaline; but a consideration of the properties of the different indicators employed fortunately clears up the problem and leaves us in possession of more definite information in the end as to the salts present than does the mere statement that the reaction is acid or alkaline.

This varying behavior of the indicators teaches us in the first place that the simple statement ought never to be made that the intestinal contents are acid or alkaline, but instead the statement that the contents are acid or alkaline *to a specific indicator*.

This remark applies to other fluids, such as saliva, bile, pancreatic juice, and blood, in which varying reactions may be obtained with different indicators. In each case the indicator used ought to be specified distinctly in stating the reaction. These fluids had until recently been universally regarded as alkaline, because the reaction had always been tested with litmus, and with this indicator they all

¹ The reaction of the intestine in man has been always determined either some considerable time after death, or in cases of fistula caused by a diseased condition of the intestine.

give a distinct alkaline reaction. It has recently been shown, however, that certain of these fluids give an acid reaction with phenolphthalein,¹ and from this point of view they have been spoken of as acid fluids, although it has been recognized that such a reaction to phenolphthalein does not indicate the presence of free acid.

In general terms, it may be stated that all the fluids of the body with the exception of gastric juice, possess at the same time an alkalinity and an acidity, because their reaction is due to combinations of the alkalies with weak acids such as carbonic and phosphoric, forming the so-called acid salts which give an alkaline reaction with some indicators and an acid reaction with others. This fact has long been appreciated in the case of the blood, in which the amount of alkalinity and acidity in the same sample has frequently been determined,² but seems to have been too much forgotten in the case of other fluids, and particularly so in the case of the intestinal contents.

In view of this difference in action of various indicators and the evidence given thereby as to the character of the substances causing the varied reactions, it will be advisable to describe in outline the behavior towards acids and alkalies, and certain salts, of those indicators which we have employed, before stating the results obtained by their application to testing the reaction of the intestinal contents. Such a brief review of the action of the indicators is made all the more essential by the fact that statements are made as to the use of these different indicators by other workers which in our opinion are inconsistent with the present state of chemical knowledge as to their action.

Thus, Gillespie states that "in the majority of the observations phenolphthalein was used as an indicator, with check estimations performed with litmus." Now litmus cannot be used to check phenolphthalein in this way, because the end point, in a fluid containing acid salts of the alkalies such as the carbonates or phosphates, would be quite different in the two cases.

Again, Chittenden and Richards conclude from the experimental facts that saliva has an acid reaction to phenolphthalein and an alkaline reaction to litmus, that the alkalinity indicated by litmus must be due to some acid salt or salts, like the hydrogen alkali phosphates, with possibly some alkali bicarbonate. Now we can confirm the reactions of saliva to phenolphthalein and litmus as stated by these authors

¹ JOLLES: *Archiv f. d. ges. Physiol.*, 1894, lxxvii, p. 1; CHITTENDEN and ALBRO: *This journal*, 1898, i, p. 317; CHITTENDEN and RICHARDS: *ibid.*, p. 462.

² For references, see SCHAEFER: *Textbook of physiology*, i, p. 145.

and we agree that this demonstrates the presence of some acid salt of an alkali; but we cannot see why the alkalinity should be due any more to acid phosphates than to bicarbonates of the alkalies; for phenol-phthalein is neutral to bicarbonate of sodium solutions, and reacts acid to these when they contain excess of carbonic acid.¹ Further, it is known from Pflüger's determinations that saliva does contain an excess of carbonic acid, — so does blood plasma and so probably do other secretions such as bile and pancreatic juice. Hence there is no reason to doubt that the alkalinity of all such fluids to litmus is due in great part to bicarbonates of the alkalies; for it is known from quantitative determinations of the ash that such fluids contain more alkali than can be apportioned to the amounts of stronger acids and phosphoric acid present. Nor does there seem much error in the common statement objected to by these authors that these fluids contain sodium carbonate, since it is well known that there is excess of carbon dioxide in solution and hence that the carbonate must be present as bicarbonate, although it might be somewhat less ambiguous to state the percentage as bicarbonate and not as carbonate in such a case.

The indicators employed in the present investigation were methyl orange, lackmoid, litmus, and phenol-phthalein.² These four indicators form a series of varying stability in which methyl orange is most stable, and phenol-phthalein least stable, while the others are intermediate in the order given above. Now the change in color of an indicator is caused by the association or dissociation of the organic acid in its molecule, and hence change in color will only be caused by acids or alkalies capable of bringing about this change. If the acid present in the indicator be stronger or more stable than the acid free in the solution the indicator will not be affected. For example, the acid present in methyl orange is stronger than carbonic acid, or oleic, palmitic, or stearic acid,³ and accordingly it will not show an acid reaction in presence of an excess of any of these acids but will remain neutral; while to salts of these acids, such as sodium carbonate and bicarbonate and the sodium soaps, it will give a strong alkaline reaction, because it forms more stable salts than these and hence combines with their bases and becomes more associated. Phenol-phthalein on

¹ *Vide infra*, p. 321.

² For a fuller discussion of the properties of these indicators, see SUTTON: Volumetric analysis, 7th ed., 1896, pp. 36-44, where references to the literature of the subject are given.

³ As well as many other weak acids.

the other hand contains a very weak or unstable acid which is readily dissociated even by the weak organic acids (such as those mentioned above) and by carbonic acid. It therefore shows an acid reaction in many cases where a more stable indicator shows an alkaline reaction.

It will accordingly be seen that we have here a series of indicators sensitive at the one end to acid radicles and at the other to alkaline radicles and by a proper use of the series, information may be obtained as to the *nature* of the acidity or alkalinity which is much more valuable than a mere statement of the reaction to one indicator such as litmus.

The behavior of these indicators towards those constituents which are most likely to be present in the intestinal contents, whether arising from the digestive juices or intestinal mucous membrane, or formed in any possible bacterial action going on within the intestine, has been specially investigated by us as a preliminary to the employment of these indicators in testing the reaction of the intestinal contents. In so testing the indicators we have endeavored as far as possible to obtain quantitative results as to the delicacy with which the indicators react to those acid or alkaline substances which are most probably present.

In the first place we determined the reaction to various free acids.

It was found that all four reacted sharply acid to *minute* traces of free hydrochloric acid, the most stable of the four, namely, methyl orange, distinctly showing an acid reaction in a solution of 1 part of hydrochloric acid in 1,000,000 of distilled water. Towards the stronger organic acids which would be set free in bacterial decomposition of carbohydrates or fats, such as acetic, lactic, and butyric acids, all the indicators were acid with the following quantitative limits.

Indicator.	Acetic acid.	Lactic acid.	Butyric acid.
Methyl orange	{ 1 in 150,000 to 1 in 200,000	1 in 600,000 to 1 in 800,000	1 in 100,000 to 1 in 150,000
Jackmold	{ 1 in 800,000 to 1 in 1,000,000	1 in 800,000 to 1 in 1,000,000	1 in 400,000 to 1 in 500,000
Litmus	{ 1 in 200,000 to 1 in 300,000	1 in 400,000 to 1 in 500,000	1 in 200,000 to 1 in 300,000

The upper figures give the amount of each acid in each case necessary to be added to distilled water to give a *distinct* acid reaction, and the lower figures give the dilution at which the acid reaction became doubtful. The tests were made by adding in each case three drops of a one per cent solution of the indicator to ten c.c. of the diluted acid contained in a test tube and comparing the tint developed, with that obtained under similar conditions in a like test tube containing distilled water. The tubes were observed either against a clear daylight sky or against a white background. Similar tests cannot be applied with accuracy in the case of phenol-phthalein since this indicator is colorless to acids. Faintly alkalized phenol-phthalein turns colorless however even with very dilute solutions of these acids, and other experiments show that it reacts to acids even more delicately than the three indicators tested above. In the case of methyl orange it may be mentioned that the solutions do not turn so red an orange as with traces of mineral acids, still the orange color obtained with the above stated traces is quite easily distinguishable from the pale lemon yellow obtained with distilled water or dilute alkali.

The reaction of the indicators towards the weak acids of the body fats was next tested by employing one per cent solutions of pure stearic and of pure oleic acid respectively in warm alcohol, and adding in each case one or two drops of one per cent solution of the indicator to four to five c.c. of the solution. Both oleic and stearic acid solutions in alcohol were found to be neutral to methyl orange and acid to the other three indicators. The stearic acid solution was nearly neutral to lackmoid and the reaction became more doubtful on dilution, while oleic acid solution was strongly acid, even on dilution. Litmus is much more easily affected by these acids than is lackmoid, and phenol-phthalein is the most sensitive of the four.

The behavior of the indicators towards dilute solutions of the so-called acid salts of the alkalies was next tested, and for this purpose solutions containing one per cent of the dried sodium salts were prepared. The salts tested were the bicarbonate, monosodic phosphate, and disodic phosphate. These salts were tested both in simple solution in distilled water and in solutions through which carbonic acid gas was passed either before or after adding the indicator.¹ The latter condition is that in which these solutions are most comparable to the intestinal contents, for there an excess of carbonic acid is always present. It is impossible to prepare a solution of sodium bicarbonate which is entirely free from normal carbonate because the

¹ The indicator was added afterwards in the case of phenol-phthalein, in order to avoid any possible error from its destruction by the excess of carbonic acid.

sodium bicarbonate partially dissociates on solution with evolution of carbonic acid gas.¹ Such a solution containing as little normal carbonate as possible is alkaline to all four indicators,² but the alkaline reaction to phenol-phthalein is very slight while marked to the other three indicators. If now carbonic acid gas be passed through the solution, it rapidly becomes acid in reaction to phenol-phthalein, the neutral point probably lying at the point at which no normal carbonate is present.³ At a later stage, the solution turns acid to litmus also, but, even with prolonged passage of carbon dioxide at atmospheric pressure never becomes acid to lackmoid⁴ or methyl orange. The demonstration of this change in reaction may easily be made by dissolving one per cent of dried normal sodium carbonate in water (a strength very much exceeding anything present in any of the body fluids) and then passing a stream of carbonic acid gas through the solution, which first turns acid to phenol-phthalein and later to litmus. To solutions of disodic phosphate in distilled water containing one per cent of the dried salt⁵ all four indicators are alkaline, but phenol-phthalein very faintly so, as the reaction changes to acid on adding *one* drop of a one per cent solution of the *monosodic* phosphate to 10 c.c. of the disodic phosphate solution. Hence the disodic phosphate may without sensible error be taken as the neutral point to phenol-phthalein. On passing carbon dioxide through the disodic phosphate solution, the difference in reaction of the four indicators is beautifully demonstrated. A few bubbles suffice to change the reaction to acid in the case of phenol-phthalein; after some minutes, the reaction becomes acid to litmus also; a still greater excess gives an acid reaction with lackmoid, while methyl orange remains alkaline no matter how long the stream of gas may be passed.

To a solution containing one per cent of dried monosodic phosphate all the indicators except methyl orange are strongly acid. Methyl orange gives an almost neutral reaction with this solution; if there be an acid reaction at all it is more difficult to distinguish than

¹ See ROSCOE and SCHORLEMMER: Treatise on chemistry, ii, pt. I, p. 131.

² The solution used was of such a strength as to contain one per cent of dried NaHCO_3 .

³ See SUTTON: Volumetric analysis, 7th ed., pp. 43, 44.

⁴ There is a *slight* change in tint of the lackmoid containing solution which becomes more purple, but it never changes completely to acid. In other words, prolonged passage of carbon dioxide gives a nearly neutral but still faintly alkaline solution with lackmoid as indicator.

⁵ Made by dissolving 2.52 grams of crystallized salt in 100 c.c. of distilled water.

those designated as doubtful in the above quantitative tests, *i. e.*, less for example than that given by one part of hydrochloric acid in one million parts of distilled water.

We have employed two distinct methods of experimentation in applying these indicators to determine the reaction of the intestinal contents in animals. In the first method, we have determined the reaction all along the intestine as rapidly as possible after death, using the method previously described by Moore and Rockwood,¹ and extending our observations to the sheep and calf.

In the second method, we have established in two dogs a fistula of the small intestine immediately above the ileo-cæcal valve and have determined the reaction to these four indicators of the fresh contents as they have escaped from the fistula. In addition, we desire to add certain observations which we have made on the nature of the contents escaping from such a fistula.

In carrying out the testing by the first method, in order to get a clear change in color of the indicator this was made faintly of the opposite reaction before mixing with the intestinal contents, after a preliminary test had pointed out the direction in which the reaction lay.

The small intestine in six sheep which had been previously fed on grain was found to be alkaline throughout to acidified methyl orange, lackmoid, and litmus, giving a strong definite reaction with each of these three indicators. On the other hand, to phenol-phthalein made slightly alkaline the reaction was faintly acid throughout the length of the small intestine.

In the calf, similar experiments were carried out excepting that in this case the animals were in a fasting condition. Here, for eight to ten centimetres below the pylorus, the reaction was acid to all four indicators, but below that level the reaction remained acid to phenol-phthalein only and became strongly alkaline to the other three indicators.

These indications of reaction prove clearly when considered in relation to what has been shown above as to the behavior of the indicators used, that *the intestinal contents contain such salts as acid phosphates and bicarbonates of the alkalis in presence of an excess of carbonic acid.*

This conclusion is confirmed by the similar results obtained in the two dogs in which fistulae had been made. The animals were under

¹ MOORE and ROCKWOOD: *Journal of physiology*, 1897, xxi, p. 376.

observation for a period of over three weeks, during the greater part of which time they were fed upon lean meat; in the last two days in one case and three in the other, the animals were fed upon milk. Both animals died suddenly at the end of the period of examination, death being due in each case to perforation of the large intestine. During the whole period in each case with the exception of the last two or three days the animals appeared quite well and ate their diet of 400—600 grams of meat with readiness. They were also exercised during the period of observation and appeared to be in normal health.

The reaction was observed daily and was found uniformly to be alkaline to methyl orange, lackmoid, and litmus, and acid to phenolphthalein, thus confirming the results obtained in other animals after death, and being opposed to the results obtained in those three or four cases of fistulae of the small intestine in man which have been recorded.

The amount of material flowing from the fistula was scanty under the meat diet, and consisted of a black, semi-fluid, homogeneous mass. The total quantity dried and weighed for three days on a diet of 600 grams of meat per diem, only amounted to 8.3 grams, *i. e.*, 2.76 grams per diem. This illustrates how completely absorption can take place in the small intestine in a carnivorous animal. Nor was the small amount due to a passage onwards to the rectum past the fistula, for no faeces were voided during the period of observation and a very small amount was found post-mortem in the large intestine. On the milk diet, the faeces changed to a flocculent, light brown colored, semi-fluid mass, which had the same reaction as in the case of meat feeding.

The chemical nature of the contents escaping from the fistulae was also investigated, as to the food stuffs and biliary constituents, and as to the presence of digestive enzymes, with the following results:—

(a) On diluting somewhat with water and filtering, no coagulable proteid, nor albumoses or peptones, nor carbohydrate could be detected by any of the usual qualitative tests, showing that in the dog absorption of the digested food stuffs is practically complete at the lower end of the small intestine.

(b) The filtered intestinal contents very rapidly dissolved and digested fibrin in alkaline solution, showing the presence of trypsin.

(c) The filtrate also rapidly converted starch into a reducing sugar and the starch reaction completely disappeared.

(*d*) Evidence could *not* be obtained by the usual tests of the presence of a steatolytic enzyme.

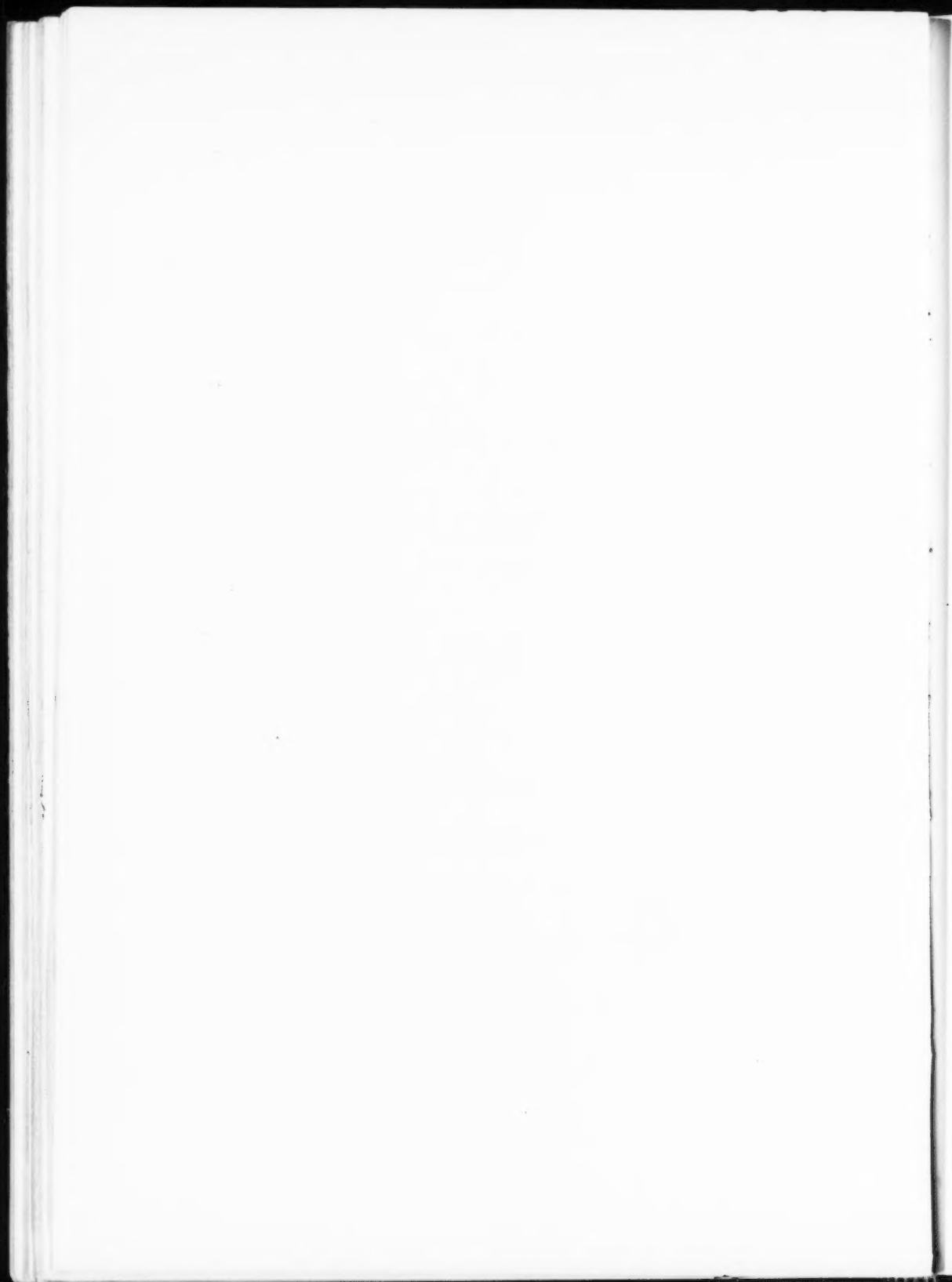
(*e*) Pettenkofer's test gave a negative or very dubious positive result, showing the absence of anything but traces of bile salts.

(*f*) Gmelin's test also gave a negative result, indicating the absence of unaltered bile pigments.

SUMMARY.

The acid reaction of the intestine to phenol-phthalein is probably due to an excess of dissolved carbonic acid. The alkaline reaction to methyl orange, lackmoid, and litmus, shows the absence of hydrochloric acid, and of all stronger organic acids, such as acetic, lactic, or butyric, which would be formed in bacterial decomposition of carbohydrates or fats.

Absorption of food stuffs, at least in the dog, can be practically completed in the small intestine. A proteolytic enzyme active in alkaline solution, and a diastatic ferment, also active in an alkaline solution are present in the dog's intestine at this level.



ON ION-PROTEID COMPOUNDS AND THEIR ROLE IN
THE MECHANICS OF LIFE PHENOMENA. I. THE
POISONOUS CHARACTER OF A PURE NaCl SOLUTION.

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I. INTRODUCTORY REMARKS ON ION-PROTEID COMPOUNDS.

IN this series of articles I intend to publish some new facts and ideas concerning the constitution of living matter and to apply these facts to a number of life phenomena. The new facts concerning the constitution of living matter are chiefly as follows: the salts or electrolytes in general do not exist in living tissues as such exclusively, but are partly in combination with proteids. The salt or electrolyte molecules do not enter into this combination as a whole but through their ions. The great importance of these ion-proteid compounds lies in the fact that by the substitution of one ion for another the physical properties of the proteid compounds change (for instance, their power to absorb water and their state of matter). We thus possess in these ion-proteid compounds essential constituents of living matter which can be modified at desire and hence enable us to vary and control the life phenomena themselves.

By making experiments on the effects of ions upon the absorption of water by muscle I found that a muscle does not take up the same amount of water in equimolecular solutions of various chlorides.¹ The differences were very striking. While in a 0.7 per cent NaCl solution the muscle absorbed about $\frac{7}{7}$ per cent of its own weight of water within eighteen hours, it absorbed about 40 per cent to 50 per cent of its weight of water in an equimolecular KCl solution. In an equimolecular CaCl_2 solution it lost about $\frac{20}{20}$ per cent of water. In a LiCl solution it neither lost nor absorbed any water. The same was true for the bromides and iodides of the same metals. Even organic compounds of these metals showed somewhat the same difference although the effects of the anion in these cases modified the results quantitatively. This difference between

¹ LOEB, J.: *Archiv f. d. ges. Physiol.*, 1899, lxxv, p. 303.

the effects of the various metal ions upon the absorption of water by the muscle shows a remarkable parallelism with the influence of the same ions upon the absorption of water in soaps. There are Na-, K-, Ca- and other soaps. While K- soaps absorb enormous quantities of water the Na-soaps absorb much less and the Ca-soaps still less than the Na-soaps. If in the Na-soap we substitute K-ions for the Na-ions the soap takes up quantities of water. If we substitute Ca-ions for Na-ions the soap loses water. From this I concluded that in the muscle the various metal ions exist in combinations in which they can as easily be substituted for each other as in the soap compounds. These compounds are similar to the soap compounds in one physical quality, namely, the absorption of water. I expressed the opinion that the ions in muscle must be in combination with proteids. If a muscle be put into a KCl solution the K-ions of the solution enter the muscle and gradually take the place of the Na- and Ca-ions in these metal proteids. The K-proteids are able to bind more water than the Na- or Ca- proteids. If the muscle be put into a solution of CaCl_2 Ca-ions will take the place of the Na- and K-ions in the proteid compounds of the muscle and the muscle must lose water. I have carried these experiments further and may publish some of the more recent results in this series of articles.

I next applied this conception of ion-proteid compounds to a phenomenon which had hitherto been observed only occasionally, namely, rhythmical contractions of the muscles of the skeleton.¹ I found that such *rhythmical contractions occur only in solutions of electrolytes, i.e.* in compounds which are capable of ionization. In solutions of non-conductors (urea, various sugars, and glycerine) these rhythmical contractions are impossible. This is an indication that they are a function of the ion-proteids. But only in certain ion solutions are such rhythmical contractions possible. All the solutions of Na-salts are able to produce them, but in a 0.7 per cent NaCl solution contractions begin later and are less powerful than in an equimolecular NaBr solution. This indicates that not only the metal ion influences the physical qualities of the ion-proteids but that the anion does so as well. This might be understood from the assumption that anions as well as kations may combine with proteids. This forces us to raise the question whether both ions may not combine with the same proteid molecule, only with the difference that the two different ions

¹ Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln bevrufen
Festschrift für A. Fick. Braunschweig, 1899.

be added at different places in the molecule. My colleague, Professor Stieglitz, with whom I discussed this question, called my attention to the behavior of amido-acetic acid, which indeed acts in a similar way. From all we know concerning the constitution of proteids it seems justifiable to assume that some of them may very well share certain peculiarities of the amido acids.¹

The experiments on the rhythmical contractions of the muscles of the skeleton, however, led to some other data concerning the ion-proteids. Solutions of Na-ions produce rhythmical contractions only if the muscle cells contain Ca-ions in sufficient numbers. As soon as there is a lack of Ca-ions in the tissues the Na-ions are no longer able to cause rhythmical contractions. On the other hand if we add Ca- or K- solutions to the NaCl solution it will no longer cause rhythmical contractions in a fresh muscle. It therefore looks as if the substitution of certain quantities of Na-ions for Ca-ions caused contractions; but if this substitution goes too far the muscle loses its irritability. On the other hand the presence of Ca-ions in the NaCl solution prevents the substitution of a sufficient number of Na-ions for Ca-ions and in the muscle thus prevents rhythmical contractions. It is due to the presence of Ca- (and K-) ions in our blood that our muscles do not contract rhythmically.

These facts received further support when I tried to determine the active alkalinity of the blood. I had found that a slight decrease in the active alkalinity of sea-water retarded the development and growth of young sea urchins while a slight increase in the number of hydroxyl ions accelerated both processes. It seemed to me that the main physiological interest in the alkalinity lay in the osmotic pressure of the free HO-ions in the blood or serum as only the free HO-ions can have any physiological effects. In the course of my experiments I found that the quantity of free HO-ions in the blood is neither increased by a considerable addition of NaHO nor decreased by a considerable addition of HCl. Experiments proved that this is not due to the salts of the blood or serum but to the proteids. It is evident that the latter have the power of combining with H- and HO-ions. Spiro came to a similar result by starting from a different point of view.² Here again we have to deal with ion-proteids.

If we look at these phenomena from a biotechnical view point they

¹ SPIRO: *Zeitschrift für physiologische Chemie*, 1899, xxviii, p. 174.

² SPIRO and PEMSEL: *Zeitschrift für physiologische Chemie*, 1898, xxvi, p. 233.

gain in significance. They teach us that we can impart to a tissue new properties by changing the quality and the relative proportions of the ions in combination with the proteids. The characteristic qualities of every tissue are partly due to the fact that its ion-proteids contain certain ions in definite proportions. Any change in this proportion is accompanied by a change in the properties of the tissue.

Ten years ago I had started upon experiments in which I substituted at desire one organ for another or transformed one organ into another (heteromorphosis). The agents I used for this purpose were various forms of contact, gravitation, and light. But the number of animals in which the phenomena of heteromorphosis could be controlled was rather limited. I concluded at that time that it would be necessary for the development of this technical or constructive side of biology to find a more elementary point of attack. The ion-proteids on account of the ease with which their properties can be changed by a change of their ions seemed to offer the desired opportunity. I therefore decided to devote last summer at Wood's Hole entirely to experiments in this direction. Marine animals which live in a medium having a high concentration of ions seemed to offer better opportunities than fresh water or land animals.

A brief report of one part of the summer's work was published in this periodical.¹ Since then Dr. W. Pauli of Vienna has published an address in which he reaches similar conclusions on ion proteids independently.² His conceptions are based on experiments upon the physical qualities of proteids. His address appeared too late to influence me in my work or my ideas, but the clearness of his results and statements were a very welcome support. He speaks in his address of ion-proteid compounds, and I only need to quote the following sentence in order to show how far his conceptions and mine agree. "We cannot doubt the general existence of ion-proteid compounds in the living organism. We have even urgent reasons for assuming that all the proteids of the protoplasm exist there only in combination with ions." I shall have a chance to discuss his views further when the full account he promises of his experiments appears.

These introductory remarks may suffice for the present. I will now

¹ LOEB, J.: This journal, 1899, iii, p. 135.

² PAULI, W.: Ueber physikalisch-chemische Methoden und Probleme in der Medizin. Wien, 1900; and Ueber die physikalischen Zustandsänderungen der Eiweisskörper, Wiener akademischer Anzeiger, 12 October, 1899.

report on a series of experiments which were undertaken on the assumption of the existence of ion-proteids, and the possibility of changing the qualities of tissues by changing the relative proportions of ions in the tissues.

II. EXPERIMENTS ON FISH.

If it be true that life phenomena depend upon the presence of a number of various metal proteids (Na, Ca, K, and Mg) in definite proportions, it follows that solutions which contain only one class of metal ions must act as a poison. The reason for this is that the one class of metal ions will gradually take the place of the other metal ions in the ion-proteids of the tissues. Even a pure NaCl solution must thus be poisonous, although this salt permeates all our tissues and is the main constituent of the inorganic matter of the ocean. I have looked through the literature in vain to find facts which corroborate this view. I found only the following data: Ringer and Locke¹ mention the fact that in NaCl solutions the contraction curve of a muscle may show slight variations, and Locke adds that the same is true for certain electric phenomena in the nerve. True has added another observation in this direction. It has been known for many years that if we put plant cells, for instance *Spyrogyra*, into a very concentrated solution of salts or sugars the cells lose water and cease to grow. True found that the osmotic pressure of a NaNO_3 and KNO_3 solution which is just able to prevent growth in *Spyrogyra*, is lower than that of a sugar solution which renders growth impossible. Hence he concludes that NaNO_3 is not harmless for *Spyrogyra*.² In this case of course the concentration of Na ions was much greater than in the blood or in sea-water. It is, however, easy to give striking illustrations of the fact that a pure NaCl solution is a strong poison. In a former paper I have shown that *Fundulus*, a marine fish, can endure an astonishing increase in the concentration of sea-water. An addition of 5 per cent NaCl to sea-water does not injure this animal.³ I selected it for testing my conceptions concerning the metal proteids. I found that it dies in a short time in a pure NaCl solution of the same concentration as that in which this salt exists in the sea-water.⁴

¹ LOCKE: Archiv f. d. ges. Physiol., 1893, liv, p. 501.

² TRUE: Botanical Gazette, 1898, p. 407.

³ LOEB: Archiv f. d. ges. Physiol., 1894, lv, p. 530.

⁴ For these experiments the young fish which had just hatched were used. As they were less than a centimetre long their mass was small compared with the volume of the solution.

In $\frac{1}{2}$ *n* solutions of NaCl which were diluted with various quantities of distilled water, the animals lived the longer the greater the dilution was, and *in distilled water* the *young fish* lived indefinitely. The following table shows the duration of life of these fish in pure NaCl solutions of various concentrations.

TABLE I.
Average duration of life of young *Fundulus* in pure NaCl solutions of different concentration.

Nature of the Solution.	Duration of Life.
100 c.c. $\frac{1}{2}$ <i>n</i> NaCl	Less than 12 hours.
90 " " + 10 c.c. distilled water	About 24 hours.
80 " " + 20 " "	" 30 "
50 " " + 50 " "	" 40 "
20 " " + 80 " "	" 60 "
10 " " + 90 " "	" 72 "
0 " " + 100 " "	Still alive after 10 days.

This result agrees with our theory. But in order to make the proof complete we must be able to show that an addition of certain other ions annihilates the poisonous effects of a pure NaCl solution. I tried the following solutions upon *Fundulus*:—

- (1) 96 c.c. $\frac{1}{2}$ *n* NaCl + 4 c.c. $\frac{1}{2}$ *n* MgCl₂.
- (2) " " " + 4 c.c. $\frac{1}{2}$ *n* KCl.
- (3) " " " + 4 c.c. $\frac{1}{2}$ *n* CaCl₂.

In each of these solutions the fish died in less than or in about 24 hours. After this experiment two salts were tried in combination with NaCl and the following solutions were prepared:—

- (1) 96 c.c. $\frac{1}{2}$ *n* NaCl + 2 c.c. $\frac{1}{2}$ *n* MgCl₂ + 2 c.c. $\frac{1}{2}$ *n* CaCl₂.
- (2) " " " + " " + " $\frac{1}{2}$ *n* KCl.
- (3) " " " + " $\frac{1}{2}$ *n* CaCl₂ + " $\frac{1}{2}$ *n* KCl.

This time the result was very striking. In the first of these three solutions the animals lived less than 30 hours, in the second a few hours longer, in the third they were still alive 10 days later when I discontinued the experiment. Thus we see that the poisonous effects of the NaCl solution really disappear if we add a small amount of K- and Ca-ions, which makes the proof of our theory complete.

The Poisonous Character of a Pure NaCl Solution. 333

In the pure NaCl solutions we have to deal with two ions, Na- and Cl-ions. Are both equally responsible for the poisonous effect? The fact that KCl and CaCl_2 prevent the poisonous effects of the NaCl solution proves that the metal ions are of greater importance than the Cl-ions.

I stated above that *Fundulus* stands the addition of rather large quantities of NaCl to sea-water. I tried to determine whether K- and Ca-ions were able to counteract even larger doses of NaCl than are contained in a $\frac{1}{8}$ *N* NaCl solution. A number of young *Funduli* were put into the following solutions:—

- | | | | |
|-----|----------|------------------------------|---|
| (1) | 100 c.c. | $\frac{1}{8}$ <i>N</i> NaCl. | |
| (2) | 96 " | " " | + 4 c.c. $\frac{1}{8}$ <i>N</i> KCl. |
| (3) | 96 " | " " | + 4 " $\frac{1}{8}$ <i>N</i> CaCl_2 . |
| (4) | 96 " | " " | + 2 " $\frac{1}{8}$ CaCl_2 + 2 c.c. $\frac{1}{8}$ KCl. |
| (5) | 93 " | " " | + 5 " $\frac{1}{8}$ CaCl_2 + 2 c.c. $\frac{1}{8}$ KCl. |

In solutions 1 and 2 the animals died in less than two hours. In solution 3 the animals were found dead the next morning. In solution 4 the animals died within three days, and in solution 5 one animal was still alive at the end of the third day when the experiment was discontinued. The presence of small amounts of K- and Ca-ions prevents or weakens the poisonous effects of even large quantities of NaCl.

I will now consider some possible objections to our theory. One might think that with the CaCl_2 a small amount of HO-ions might possibly be introduced in cases in which the CaCl_2 was heated before it was dissolved. One might think that these HO-ions were the essential constituent that prolonged the life of these fish. The K-ions were only needed to overcome certain effects of the Ca-ions. There is indeed an antagonism between the K- and Ca-ions, as shown by Ringer's experiments. But the fact that *Fundulus* lives indefinitely in distilled water proves that HO-ions are not necessary to maintain its life. The second objection might be that the NaCl used contained impurities. But this objection may be discarded at once. The NaCl was obtained from several leading factories and was chemically pure. The only impurity possible could have been a trace of K. But as a further addition of K made the NaCl more harmless it is out of the question that the trace of KCl which the NaCl might have contained could have had anything to do with the poisonous effects. A third possible objection might be that these experiments only prove the necessity of K- and Ca-ions for *Fundulus*. But this idea is refuted by the fact

that *Fundulus* can live indefinitely in distilled water. It is, perhaps, worthy of mention that the positive proof for the poisonous character of a pure NaCl solution would not have been possible except in a marine animal like *Fundulus* for which distilled water is not poisonous. Another possibility might have been the presence of a trace of acid. My colleague Professor Stieglitz was kind enough to test the NaCl used, for acids, but it was found to be absolutely free from acids. Hence I do not see any other possible explanation of the results than the theory from which we started.

What is true for pure NaCl solutions is, of course, still more true for equimolecular pure solutions of KCl and CaCl_2 . They act like poisons. I have not yet been able to convince myself that their poisonous effect can be prevented by the addition of small amounts of other metal ions.

The fact that *Fundulus* can be thrown from sea-water into distilled water without any considerable swelling, or without any visible injurious effects, may find its explanation through the influence that various ions have upon the absorption of liquids. The above mentioned experiments on the absorption of liquids by the muscle have shown that the simple osmotic theory of absorption which has been accepted by botanists cannot possibly be correct. I shall deal with this problem in another paper.

III. EXPERIMENTS ON JELLY FISH (GONIONEMUS).

The locomotion of medusæ is due to rhythmical contractions of their swimming bell. I experimented on a form which is very abundant at Wood's Hole, *Gonionemus*. If we put a *Gonionemus* into a $\frac{1}{2}$ *N* solution of NaCl it soon stops contracting rhythmically. Too many Na-ions take the place of Ca- and K-ions and this alters the physical properties of the tissues to such an extent that no more contractions are possible. If such a medusa is brought back into normal sea-water it begins to beat again after a short time. In this case Ca- and K-ions take the place of some of the Na-ions in the ion-proteids, and this restores the irritability (or contractility) of the medusa. We thus see again that the Na-ions in a pure NaCl solution are poisonous. If this idea were correct we should expect that in a more diluted NaCl solution the medusa would be able to contract much longer. This is indeed the case. I tried the following solutions:—

90 c.c.	$\frac{1}{2}$ <i>N</i> NaCl	+ 10 c.c.	distilled water.	
80	"	"	+ 20	"
70	"	"	+ 30	"
				etc.

The result of the experiments was that the *Gonionemus* contracts longest in a mixture of equal parts of a $\frac{1}{2}$ N NaCl solution and distilled water. The case is parallel to that of *Fundulus* with the exception that the *Gonionemus* is not able to stand distilled water. Otherwise the NaCl is the less poisonous for *Gonionemus* the more dilute it is. We are forced to conclude that if we add certain other metal ions to the NaCl solution its poisonous effects must disappear.

We tried the following solutions:—

- (1) 96 c.c. $\frac{1}{2}$ N NaCl + 4 c.c. $\frac{1}{2}$ N $MgCl_2$.
- (2) 96 " " + 4 c.c. $\frac{1}{2}$ N KCl.
- (3) 96 " " + 4 c.c. $\frac{1}{2}$ N $CaCl_2$.

In the first two solutions the medusæ make a few contractions during the first minute and then stop. In the third solution the rhythmical contractions may go on for an hour. If we take 2 c.c. of the $CaCl_2$, KCl, and $MgCl_2$ solution (instead of 4 c.c.) the results are practically the same. After this the following solutions were tried:

- (1) 96 $\frac{1}{2}$ N NaCl + 2 c.c. $\frac{1}{2}$ N $MgCl_2$ + 2 c.c. $\frac{1}{2}$ N KCl.
- (2) " " + " " + 2 c.c. $\frac{1}{2}$ N $CaCl_2$.
- (3) " " + " $\frac{1}{2}$ N KCl + 2 c.c. $\frac{1}{2}$ N $CaCl_2$.

In solution 1 no contractions occurred while in solutions 2 and 3 regular contractions set in, whose period was almost normal. They lasted an hour or more. Mg-ions act in this case more like K-ions. We thus see again that for the medusa the same is true as for *Fundulus*. Na-ions are poisonous in a pure NaCl solution while the same solution is harmless if a certain amount of K- and Ca-ions are present. Our theory that the irritability of tissues depends upon the presence in definite proportions of Na-, K-, and Ca-ions is once more verified. It goes without saying that a pure $\frac{1}{2}$ N KCl and a pure $\frac{1}{2}$ N $CaCl_2$ solution was still more poisonous than a $\frac{1}{2}$ N NaCl solution.

IV. EXPERIMENTS ON CILIARY MOTION.

The conditions for ciliary movements were studied in the young larvæ (blastula, gastrula, and pluteus) of the sea urchin. The movements of these larvæ are due to cilia which are incessantly active. I found that these larvæ do not die rapidly in a $\frac{1}{2}$ N NaCl solution. They may live 24 hours. But if we add a small amount of $\frac{1}{2}$ N KCl and $\frac{1}{2}$ N $CaCl_2$ they may be kept alive and in motion for to days or more. In the latter solution their development can continue, while in the pure NaCl solution this is not possible.

I was, however, surprised to find that this ciliary motion continued in solutions in which no muscular contractions of *Fundulus* or *Gonionemus* were possible. Larvæ which were 20 hours old were able to swim for about 48 hours in the following solutions:—

90 c.c.	$\frac{1}{8}$ <i>N</i> $MgCl_2$	+ 10 c.c.	$\frac{1}{8}$ <i>N</i> $CaCl_2$
80	"	+ 20	"
50	"	+ 50	"

The reader will notice that these solutions contain no NaCl. I tried the effects of these solutions on *Gonionemus*. As was to be expected not one contraction was possible in these solutions. The following observation is equally astonishing. Combinations of NaCl and KCl were tried:—

(1)	80 c.c.	$\frac{1}{8}$ <i>N</i> NaCl	+ 20 c.c.	$\frac{1}{8}$ <i>N</i> KCl
(2)	20	"	+ 80	"

In both solutions the ciliary motion continued for more than six but less than eighteen hours. The first solution seemed to be a little more harmless than the second. Anybody who has had experience with the effects of K-ions on muscular contraction will realize that it is out of the question to expect a muscle to keep its contractility for over six hours in any of these solutions. Neither was *Gonionemus* able to contract in these solutions. These experiments certainly warn us against taking it for granted that the mechanics of protoplasmic motions is the same everywhere, although there may be some identity up to a certain point. In the case of the blastula we have to deal with very young embryonic tissue, and we shall see in one of the subsequent publications that embryonic tissue, or rather the egg cells, differ radically from the muscles and the ganglia as far as the effects of ions are concerned.

V. ARE THE NA-IONS OF OUR BLOOD AN INDIFFERENT SUBSTANCE?

A pure solution of NaCl (of about 0.7 per cent) has been called the physiological salt solution, inasmuch as the tissues of a frog may live in such a solution for forty-eight hours. The NaCl in our blood is considered to play chiefly the rôle of preventing the tissues from losing or taking up any water.¹ According to our opinion the Na-ions of the blood as well as of the sea-water are essential for the maintenance of life phenomena. A reduction of the Na-ions in the blood would lead to a loss of Na-ions and a substitution of

¹ HOWELL: This journal, 1898, ii, p. 47.

other ions in their place in the ion-proteids of the tissues. But does the fact that a frog's muscle can live for about forty-eight hours in a $\frac{1}{4}$ *n* NaCl solution without being poisoned, not indicate that a pure NaCl solution is harmless for the muscle? A $\frac{1}{4}$ *n* NaCl solution is certainly not so poisonous as a $\frac{5}{8}$ *n* solution of the same salt. A *Fundulus* that would be killed by a $\frac{5}{8}$ *n* NaCl solution in twelve hours is able to live in a $\frac{1}{4}$ *n* NaCl solution two or three days, which is as long or a little longer than the frog's muscle lives at the same (summer) temperature in a solution of the same concentration. That the effects of a pure NaCl solution upon the frog's muscle are in no way different from those on *Fundulus* is proved moreover by the fact, that the muscle lives longer in a NaCl solution if small amounts of KCl and CaCl_2 are added. This explains the superiority of Ringer's solution over a physiological salt solution. Ringer's solution prevents the Na-ions of the physiological salt solution from taking the place of the Ca- and K-ions in the tissues. Contractility is only possible if the Na-, Ca-, and K-ions exist in definite proportions in the ion-proteids. Hence there is no reason for supposing that what we have proved for marine animals does not hold good for other animals.

SUMMARY.

The main results of this paper are as follows: —

1. A pure solution of NaCl of the same concentration as sea-water is a strong poison for many (if not all) marine animals. The poisonous effects of this solution are due to the Na-ions. The same is true for pure equimolecular solutions of CaCl_2 and KCl.
2. The poisonous effects of the Na-ions are antagonized by the addition of a small amount of Ca- and K-ions. Through the presence of these two ions the Na-ions in the ocean lose their poisonous effect.
3. The Na-ions of the blood would not allow the tissues to live. The presence of Ca-, K-, and possibly other ions, counteracts the poisonous effects of Na-ions in the blood. This is the reason why tissues live longer in Ringer's solution than in a physiological salt solution.
4. The reason for all these peculiar effects of the Na-, Ca-, and K-ions is that these (and other) ions form combinations with the proteids of the protoplasm. The various metal-proteids show various physical qualities. Muscles are only contractile as long as they

contain all three classes of ions in a certain proportion, which, however, may vary within certain limits. In a pure solution of NaCl Na-ions will gradually take the place of the Ca- and K-ions in the ion-proteids of the tissues, and this leads to a loss of contractility or irritability. This is the reason why a pure NaCl solution is poisonous. For the same reason pure equimolecular solutions of other chlorides are also poisonous.

5. The conditions for the ciliary motion of the larvæ of the sea urchin are in various respects different from those mentioned above. The ciliary motion of these organisms can continue for several days in a solution of $MgCl_2$ and $CaCl_2$ which is free from Na-ions.

OBSERVATIONS ON THE DEGENERATION AND RE-
GENERATION OF MOTOR AND SENSORY NERVE
ENDINGS IN VOLUNTARY MUSCLE.

BY G. CARL HUBER.

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IN this contribution it is my purpose to summarize briefly the results of a series of experiments undertaken with a view of studying the processes of degeneration and regeneration of motor and sensory nerve endings in voluntary muscle, after experimental severance of the muscular nerves.

After some experimentation, the posterior tibial nerve was selected as being the nerve most suitable for this series of experiments. This nerve gives motor and sensory nerve supply to the interossei metatarsi, which in the rabbit are relatively large and made up of many short muscle fibres. As each muscle fibre has its motor ending, in well stained preparations of the interossei muscles many such endings would be found in each field of the microscope. It was further found that the neuro-muscular and neuro-tendinous spindles were prevalent in these muscles, thus making it possible to carry on observations on the three kinds of endings at the same time. The posterior tibial was exposed by making an incision in front of the *tendo Achillis*, at a distance varying in the several experiments from two to five cm. above the os calcis. In this region the nerve is so superficial that it may be exposed through a small skin wound which heals readily by first intention. The method of procedure in each experiment was as follows:—

After exposing the posterior tibial nerves on both sides, they were crushed by means of an instrument devised for the purpose. This little instrument, which has proved very useful for this purpose, was made from a pair of artery forceps. The teeth on one side were filed away, leaving a smooth surface, and the other blade was shaped into a blunt wedge. By slipping the flat blade under the exposed nerve and pressing the wedge down upon the nerve by closing the forceps, the nerve was effectually crushed. This procedure proved much simpler than crushing the nerve by ligature. After crushing the

nerve it was stimulated above the point of crushing with the induced current to see whether its conductivity had been interrupted; if so, the skin wound was closed by silk sutures. After the expiration of the time set for the experiment the nerve was again exposed, and stimulated with the induced current above and below the point of crushing, and the results noted. The wound was then again closed, the femoral artery exposed, a cannula inserted, and a sufficient quantity of one per cent solution of methylene blue in normal salt injected to give the lower part of the leg and foot a decidedly blue color. After the expiration of some twenty to thirty minutes, the interossei muscles and the posterior tibial nerve to the point of crushing were exposed and removed to a slide moistened in normal salt, where they remained until, on examination under the microscope, the nerve fibres and nerve endings seemed well stained. The tissues were then either fixed in ammonium picrate and mounted in ammonium-picrate-glycerine, or were fixed in ammonium molybdate, sectioned on the freezing microtome, dehydrated, and mounted in balsam.

The following statement as to the reliability of the *intra vitam* methylene blue method for staining the nerves and nerve endings and the changes which they undergo in degeneration and regeneration may not be out of place, since the value of the observations to be given depends largely on this factor. The method is too well known to question the positive results obtained. It is true that in the most successful preparations obtained, only the neuraxes of the nerve fibres and their arborizations are stained. Yet the observer has no difficulty in distinguishing between neuraxes of medullated nerve fibres and varicose, non-medullated nerve fibres, and in locating the nodes of Ranvier in the former. In a degenerating medullated nerve fibre the myelin stains more readily than in a normal fibre so that the segments of a degenerating medullary sheath may be made out. On the other hand no definite conclusion may be based on the absence of stained nerve fibres or nerve endings, nor on a partial staining of the same, nor on the fact that certain nerve fibres or nerve endings stain more deeply than others. The precariousness of the method is such that in normal tissue nerve fibres and nerve endings remain now and again wholly unstained or are only partially brought to view. My conclusions are based on the positive results obtained.

In all, fifty-seven rabbits were operated upon on both sides. The

time elapsing between the crushing of the nerves and the injection of the methylene blue varied from twenty-three hours to six days for the animals used for observing the degenerative processes in the injured nerve and from twenty-eight days to one hundred and seventy-eight days for the observations on the regeneration of the nerves and nerve endings.

Degeneration of the motor and sensory nerve endings.—In experiments in which the posterior tibial nerve was stimulated with the induced current below the point of crushing, at times varying from twenty-three to forty-six hours after crushing the nerve, and this procedure followed by the injection of the methylene blue, the fact is brought out that the response of the muscles to electrical stimulation of the nerve disappears with the appearance of noticeable structural changes in the motor endings and the adjacent medullated motor nerves.

Till the end of the first day after crushing the posterior tibial nerve, practically all the motor nerve endings in the interossei muscles present a normal structure and the muscles respond to an electrical stimulation of the nerve. During the second day noticeable structural changes are seen in the motor endings. These are ushered in by relatively large, usually round or oval thickenings, varying in number, size, and shape, which appear on the arborizations of the motor endings. These enlargements stain very deeply so that in methylene blue stained preparations such endings may readily be recognized even under low magnification. The changes do not affect all the motor endings at the same time, a varying number of apparently normal motor endings being found toward the end of the second day; here and there a normal motor ending may now and then be seen even after electrical stimulation fails to excite a contraction of the muscle. Toward the end of the second day after crushing the nerve,—the time varies within several hours in the several experiments,—the medullary sheaths of the extreme distal portion of the medullated motor nerves begin to segment, showing that these portions of the motor nerve fibres are in the first stages of degeneration. The response of the muscle fibres to stimulation of the posterior tibial nerve disappears when the great majority of the arborizations of the motor nerves show the nodular enlargements here mentioned and the extreme distal portions of the motor nerve fibres show a segmentation of the myelin. That the extreme distal end of a severed motor nerve and its end-arborization degenerate

before the remaining portion of the nerve distal to the point of crushing degenerates I have observed in a number of experiments in which I was able in methylene blue preparations to trace normal medullated nerve fibres seen in small muscular branches into medullated nerve fibres showing a segmentation of the myelin and very nodular end-arborizations. The degenerative changes which follow the segmentation of the myelin and the formation of nodular enlargements on the end-arborizations, above mentioned, are not clearly brought out by the staining method used, since such fibres and endings fail at this time to respond with any degree of certainty to the methylene blue reaction. The observations made for this period may be summed up in the statement that the arborizations disappear or at least fail to stain differentially; at times the so-called sole plate stains a faint blue. The further degeneration of the motor nerve fibres will not be considered in this account.

During the latter part of the second day and the beginning of the third day after crushing the posterior tibial nerve, relatively large, normal axis cylinders are found here and there in the smaller nerves of the interossei muscles. In a number of my preparations I was able to trace such axis cylinders to normal or nearly normal sensory nerve endings, both neuro-muscular and neuro-tendinous spindles. I conclude, therefore, that the extreme distal portion of severed motor fibres and their endings degenerate earlier than do the extreme distal portion of the sensory nerves and their endings found in the same nerve trunk. This is contrary to Bethe's recent observations, who states that in a nerve trunk the continuity of which has been severed, the sensory fibres begin to degenerate somewhat earlier than do the motor nerve fibres. Toward the end of the third day, sometimes earlier, the myelin surrounding these larger axis cylinders shows segmentation and the nerve endings in the sensory end-organs break up into irregular, deeply staining fragments which gradually disappear.

Regeneration of motor and sensory nerve endings. — My observations go to show that under favorable conditions there may take place a complete regeneration of the motor endings and the nerve terminations in the complicated neuro-muscular and neuro-tendinous end-organs of muscular nerves previously degenerated. The experiments of this series, varying in time from twenty-eight to one hundred and seventy-eight days, show that a muscle which fails to respond to electrical stimulation of its nerve, degenerated by reason of an

interruption of its continuity, again responds to electrical stimulation as soon as fully developed motor nerve endings may be shown in the muscle with suitable stains. In no instance was I able to excite contraction of the interossei muscles by stimulation of a posterior tibial nerve previously degenerated, until such time as I was able to find a certain proportion of normal motor endings in the interossei muscles. Scattered motor endings were found in the interossei muscles thirty days after crushing the posterior tibial nerve. At this time many small, varicose nerve fibres were observed in the small intramuscular nerve bundles, and now and then such small varicose nerve fibres could be traced to muscle fibres where each terminated either in a small granule or in two or three small terminal branches, each of which ended in a granule. The granule I took to be the first indication of the end-arborization of regenerating motor nerves, for intermediary stages between this and fully developed arborizations were found. My observations on the relation of the regenerating axis cylinders to the sheaths of the degenerated intramuscular motor fibres, and on the relation of the new arborizations of the regenerating nerves to the muscle fibres, are not completed. It would appear, however, that the regenerated motor endings are in every respect like the motor endings found before degeneration. The regenerated motor nerve endings appear earliest in the first or innermost of the interossei muscles, a little later in the second, and by the fortieth day in all the interossei muscles.

The regenerating nerve terminations in the neuro-muscular and neuro-tendinous end-organs are as a rule not readily stained nor easily recognized until they present a structure which does not differ materially from that of the normal endings. I have observed in a number of instances an axis cylinder, ending in a neuro-tendinous spindle, divide soon after entering the spindle into a number of varicose branches, some of which could be traced to the extremities of the spindle without further branching; other branches gave off a varying but small number of short side branches which terminated in irregular end disks of varying size and shape. I have thus far not observed what may be regarded as the first appearance of the nerve terminations in the neuro-muscular spindles. All my preparations thus far have shown a spiral arrangement of rather large terminal branches of axis cylinders traced to neuro-muscular spindles.

In an experiment in which the animal was killed forty-one days after crushing the posterior tibial nerves, in which regenerated motor

nerve endings were found in all the interossei muscles, I observed one tendon spindle in the earlier stages of regeneration. It is not, however, until the end of the second month or the beginning of the third month after crushing the nerve that the nerve terminations in the neuro-muscular and neuro-tendinous spindles present an appearance similar to that found in these end-organs in their normal state.

CONCLUSIONS.

1. The motor and sensory nerve endings of voluntary muscle degenerate after severance of the muscular nerves. The motor nerve endings and the extreme distal portion of the motor nerves degenerate earlier than do the sensory nerve endings.

2. The motor and sensory nerve endings in voluntary muscle may under suitable conditions regenerate completely. The motor nerve endings regenerate more quickly than the sensory nerve endings.

3. These experiments, it seems to me (although this is not emphasized in this report), show clearly that the regeneration of a degenerated portion of a peripheral nerve and its termination is brought about by the down growth of the axis cylinder of the central, undegenerated portion of the nerve fibre.

Further observations in this field are now in progress.

A STUDY IN THE HELIOTROPISM OF CYPRIDOPSIS.

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GRANT¹ in 1829, in the course of some notes on the effect of light on Medusæ, made the following observation: "From the soft gelatinous texture of such beings, it seems natural to expect that an agent impinging on them with such velocity and in so great a quantity as the rays of light, and which penetrates their whole substance, should be able to affect them in some manner, were it only with impressions of touch." "The physical distribution of the lowest species of fixed and nerveless animals is principally determined by the intensity of light." This, with the exception of Trembley's observations on Hydra in 1744, is the earliest suggestion that I have found of the importance of the influence of light on animal forms. But workers in physiology have been very slow to study the problems here suggested; and not until quite recent years has any careful work been done in this direction.

Bert in 1868² and Lubbock in 1884³ made a number of experiments to determine the "color preferences" of different forms; but in this work they took into consideration only wave lengths, color as such, leaving out other important factors, such as the intensity and direction of the light rays. In 1883 and 1884 Graber⁴ made an exhaustive series of experiments in which he attempted to solve the problem whether and in how far animals are in a condition to distinguish intensity and color differences. He made a distinct advance on his predecessors in that he considered the influence of varied

¹ GRANT, R. E.: Edinburgh journal of science, 1829, x, p. 347.

² BERT, P.: Mémoires de la société des sciences physiques et naturelles, Bordeaux, 1868, vi, pp. 375-383.

³ LUBBOCK, J.: International scientific series, 1884, xlii, New York.

⁴ GRABER, V.: Sitzungsberichte der kaiserl. Akademie der Wissenschaften zu Wien, 1883, lxxvii, pp. 201-236; Grundlinien zur Erforschung des Helligkeits- und Farbensinnes der Thiere. Pr.g, 1884; Sitzungsberichte der kaiserl. Akademie der Wissenschaften zu Wien, 1885, xci, pp. 129-150.

intensity as well as of different wave lengths. But he, too, ignored entirely the third and most important factor, the *direction* of the light rays.

In the mean time a great deal of work had been done by Cohn, Engelmann, Wiesner and others on plants. Most significant of all was the paper published in 1878 by Strasburger.¹ He had experimented on many different kinds of swarm spores, and reached the conclusion that the movement of an organism in response to light takes place, in the majority of cases, in the direction of the rays. Under the influence of light, he maintains, an organism so orients itself that its main axis is parallel to the incoming rays, and the subsequent direction and definiteness of its movement towards or from the source of light are determined by the intensity of these rays.

This explanation of the reactions of these motile plant spores as purely mechanical phenomena was so simple and so reasonable that it seems strange that for twelve years no one applied it to animal forms. Bert, Lubbock, and Graber had all found it necessary to introduce a psychic element in order to explain their results, and it was left for Loeb² to show that the laws which Strasburger formulated for plant movements are the same as those which govern animal reactions. An animal, he states, so orients itself that its median plane lies in the direction of the impinging rays, and its movement is therefore in this direction. The precision of its orientation varies with the intensity of the light. "Light of unvarying intensity acts on animals as a constant heliotropic stimulus. The dependence of animal movements on light is point for point the same as the dependence of plant motion on this source of stimulation."³

The older idea of a response more or less psychical survives in a measure in the school led by Verworn and Oltmanns. They maintain that all light reactions are the result of an effort on the part of the organism to reach a certain optimum intensity. This explanation

¹ STRASBURGER, E.: *Wirkung des Lichtes und der Wärme auf Schwärm-sporen*, Jena, 1878.

² LOEB, J.: *Verhandlungen der physikal.-medizin. Gesellschaft zu Würzburg*, 1888, pp. 1-5; *Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen*, Würzburg, 1890; *Archiv f. d. ges. Physiol.*, 1890, xlvii, pp. 391-416.

³ "Das Licht wirkt bei constanter Intensität dauernd als heliotropische Reizursache auf die Thiere. . . . Die Abhängigkeit der thierischen Bewegungen vom Licht ist Punkt für Punkt die gleiche wie die Abhängigkeit der pflanzlichen Bewegungen von derselbe Reizursache."

is, it seems to me, made improbable by the more careful investigations of Strasburger, Loeb, Driesch,¹ and others. Certain observations also which I have made lead me to agree with Loeb. One point, I believe, I have been able to add, namely, that the external conditions of light intensity and temperature remaining constant, other factors may, in some cases at least, intervene to change the character of the response.

The experiments to be described were made in the Biological Laboratory at Bryn Mawr College during the winter of 1898-99, under the direction of Professor T. H. Morgan, to whom I wish to express my indebtedness for kindly suggestion and criticism.

I decided to investigate the problem of light response in Ostracods, as little work had been done on this group, and *Cypridopsis vidua* var. *obesa* was chosen, because it was found to be more sensitive than any other species that could be obtained. It was also large enough so that the movements of isolated individuals could be followed. The experiments may be classified under three main divisions:—

- I. Experiments in gaslight.
- II. Experiments in diffuse daylight.
- III. Experiments in direct sunlight.

I. EXPERIMENTS IN GASLIGHT.

Preliminary experiments were made with large numbers of individuals, brought from the aquaria into round shallow glass dishes which were painted black inside, in order to eliminate all possibility of reflection. But the results were so irregular and indefinite that it was found necessary to adopt some other method.

The apparatus finally decided upon was as follows: a tin trough (Fig. 1, A), painted black inside, ten inches long, $\frac{1}{2}$ inch wide and $\frac{1}{4}$ inch deep; a gas lamp B, Argand or Welsbach, placed 12 inches from the nearer end of the trough, and at an elevation of $6\frac{1}{2}$ inches, the arrangement being similar to that used by Davenport² in his experiments on *Daphnia*. Between

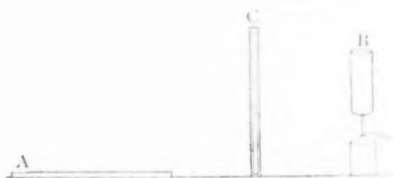


FIGURE 1.

¹ DRIESCH, H.: Zoologische Jahrbücher, 1898, v. p. 147.

² DAVENPORT, C. B.: Experimental Morphology, Part I, New York, 1897, p. 204.

the lamp and the trough was fastened a screen C, consisting of a thin glass case containing water which was changed frequently. The entire apparatus was then placed under a hole in the ceiling, connected with a fan, by which the heat radiated from the lamp was drawn away, thus preventing any warming of the air in the room and consequent slow rise in temperature of the water in the trough. Black screens were placed around the trough to cut off the diffuse light of the room. Observations on the temperature of the water in the trough before and after the experiments showed usually that it varied very little.

Animals were taken from the aquarium and placed in glass evaporating dishes, from which they were to be moved one by one by means of a pipette into the trough. But I observed that if the individuals to be used stood exposed to the gaslight while others were being experimented on, they seemed later to have lost much of their sensitiveness. It became necessary, therefore, either to bring them in one by one from outside the dark room, or to keep them in a dark dish, taking out one as needed for the experiment. These two methods gave similar results, and the latter was chosen as more convenient.

Three different intensities of gaslight were used: the first, an Argand lamp turned low; second, the same raised to its full height, and third, a Welsbach lamp turned high. These were approximately in the proportions of 1:2:8.

The method of experiment was as follows. The trough was filled with water from the aquarium and its temperature recorded. A *Cypridopsis* was then taken with a pipette from the black dish and dropped into the middle of the tin trough. The direction of its first response, toward or from the light (+ or -), and the number of seconds taken for it to reach one or the other end, were noted. It was then given sixty seconds in which to change its position. If it remained at the end reached during this time, it was removed and the experiment repeated on another individual. If, however, it left this end before the sixty seconds passed, the length of its stay there, the character of its next response, + or -, and the time taken in any further reactions were noted. If in any case the individual showed no definite response, not reaching either end but wandering aimlessly about or remaining quiet in the middle of the trough, this was indicated by a ? or 0. To illustrate, I copy the following series of observations from my note-book: —

Temp. 19° C.

Time.	No. of Experiment.	Sense of Response.	No. of secs. to reach end.
12.50 P.M.	1	+	35 ⁰⁰
	2	0 +	80
	3	+	55
	4	+	50
	5	+	30
	6	+	35
	7	—	65
	8	+ 0	
	9	—	25
	10	+ —	50, 40
3.50 P.M.	1	+ — +	20, 65, 70.
	2	+	25
	3	+	37
	4	+	25
	5	+	25
	6	+	60
	7	0	
	8	+	35
	9	0	
	10	+ ?	

In this way a very large number of observations were made, about eighty for each light intensity. Averages are given in the next table.

No. used.	Light intensity.	No. $\frac{1}{2}$ +	No. $\frac{1}{2}$ -	No. $\frac{1}{2}$ \pm	No. $\frac{1}{2}$ or 0.	Average time, $\frac{1}{2}$ +	Average time, $\frac{1}{2}$ -	% of \pm , 1st +	Longest time, $\frac{1}{2}$ +	Longest time, $\frac{1}{2}$ -	Shortest time, $\frac{1}{2}$ +	Shortest time, $\frac{1}{2}$ -
85	1	42 = 49%	18	20 = 23%	5	43"	40"	60	100"	100"	20"	20"
80	2	42 = 52%	6	25 = 31%	6	35"	43"	48	90"	60"	20"	25"
80	3	34 = 42%	8	29 = 36%	9	36"	45"	86	70"	80"	15"	18"
245		118 = 48%	32	74 = 30%	20							

¹ Individuals which changed from one response to the other. ² That is, time taken to go from one end of the trough to the other.

In most cases the animals had been in diffuse daylight, as the aquarium was kept by a west window into which little sun found its way in the winter. But in the earlier experiments a careful record of previous conditions was not kept.

Examining these averages a few points are to be noted.

1. In general the time of response is shorter in the stronger light, though the difference is too slight to warrant one in drawing any inferences from it.

2. The proportion of those giving no definite response (?) or no response at all (0), is comparatively small.

3. The number of those giving both responses (\pm), and of these the proportion in which the first response is positive, is large.

The thought suggests itself that if the positive individuals had been left long enough, they too might subsequently have given a negative response.

The significance of these results being far from clear, further experiments were made to see if any clue could be obtained as to the cause of the irregularities. Apparently the same individuals could be now positive, now negative.

In order to determine whether the response of an individual was due either to the time of day at which the experiment was made, or to a definite period in its own life, a num-

ber were experimented on as above, and sorted into three dishes, +, -, and \pm . At a different time on the following day these were tested again and others added. These again were re-sorted the next day and so on for several days in succession. But it was found that the results were quite irregular. Those with a given response at one time might give any other when tried again, though in the majority of cases here as before, the response was positive, or positive followed by negative. It thus became evident that the reaction of any one individual was not constant, but might vary at least from day to day. It might be objected that in dropping the animals into the trough, I unconsciously gave them an impetus in one direction or the other; but this error was carefully avoided either by dropping them in vertically or by putting the end of the pipette under water and letting them swim out as they would.

The next experiments¹ were made to determine whether the response of an individual was affected by merely picking it up. A Cypridopsis was taken from the dish with a pipette, dropped into the trough, and its first definite response noted. It was then picked up and again dropped in; this was repeated a number of times and each response noted. I cite one such series of observations:—

1. + + + + +	6. + + + + -
2. + + + + +	7. + + - + +
3. + + + + +	8. - + + + +
4. + + + + +	9. + + + + +
5. - + + + +	10. + + - - + - + + + +

In almost all cases the first response was positive and where doubtful at first it became steadily positive after the animal had been several times disturbed. These results suggested the following series of experiments which served to harmonize some of the inconsistencies at first noticed.

The same apparatus as before was used, with the addition of another water screen at the opposite end of the trough.² The method

¹ In these and all following experiments in the dark room, the Welsbach lamp was used.

² The range of temperature within which the following observations were made was from 15° to 19° C.

was to drop a Cypridopsis, as before, into the middle of the trough and to note its first response as soon as well defined, but before it reached the end of the trough; the light was then quickly changed to a corresponding position at the other end, being moved directly over, and in a line parallel with the trough. If the animal turned in its course when the light was moved, its response remained positive or negative as before; if it continued its course *without change*, its response was noted as the opposite of that preceding. In this way the light was changed a number of times without the Cypridopsis ever being allowed to reach the end of the trough. After some time it was permitted to reach the end and to swim about as it would. If then it turned and started down the trough, in a direction, with regard to the light, *opposite* to its former course, the lamp was again moved as before to determine whether this new direction of movement represented a distinct reaction to the light, or whether it was purely accidental. In almost all cases a very definite result was obtained; the first response was retained for some time by constantly shifting the light, the animal turning with each change and never touching the end of the trough. If then it was allowed to reach the end and to swim about there, it sooner or later turned and moved down the trough in the opposite direction. If the lamp was again shifted from end to end, this new response — *the opposite of the preceding* — persisted in a similar manner. If, for example, the Cypridopsis was at first positive, *i. e.* moved toward the source of light, — and this was generally the case, — if the lamp was moved the animal turned about in its course and swam again toward the light; if moved again, it turned again, and so on, *provided always that it was never given time to reach either end*. If now it was allowed to come to the end and left to swim about there, sooner or later according to the individual, and in a certain degree according to previous conditions,¹ it turned about and moved down the trough *away from the light*. If then the light was shifted from end to end as before, this negative response persisted, as had the positive before. If, finally, the negative end of the dish was reached, in the same way a subsequent change to positive followed.

In a few cases the change from positive to negative (never from negative to positive) occurred soon after the animal was dropped in and before it touched the end. But such instances were rare. Usually the change from positive to negative took place in less time than that

¹ See pp. 358 and 363.

from negative to positive,¹ so that if a number of individuals were put in the dish at once, there was a gradual accumulation at the negative end. The changes from positive to negative and back again could be repeated any number of times on the same individual. The \pm individuals in the earlier series of experiments were simply those in which the first change took place in less than sixty seconds.

In this same connection another phenomenon was observed. It has been suggested that the stimulus of picking up the animal may be one factor in causing its first positive response. It was found that if an animal in the negative condition, if we may so call it, *i. e.* in its course down the trough away from the light, was picked up with the pipette and dropped in again, either in the same spot from which it came or in any other part of the trough, it became positive, *i. e.*, it moved toward the light. In the majority of cases this change took place after once picking up, although sometimes an individual was taken up a number of times in succession before a permanent change was effected, that is, before a distinct positive response followed.² Different sized pipettes were tried, but the same result was obtained. It was also found that sufficient agitation of the water alone would make the individuals positive, but in no case did I succeed in making a positive animal negative in this way. If the animals be shaken when brought to the light, the very great majority are at first distinctly positive, though many soon become negative.³

In seeking an explanation for these phenomena, one idea that suggested itself was that as the trough was made of tin and the ends cast a small shadow, the change at the ends might be due, in part at least, to the influence of the shadow. To obviate this difficulty pieces of cover-slips were placed obliquely at the ends in such a way that the animals could not get into the shadow. The *Cypridopsis* then swam to the surface of the water, up the incline of the glass, but the same changes took place as before. I tried also screening parts of the trough from the light, but found that the shadows alone did not change the character of the response, though if they were long enough they

¹ This latter change occurred only after long intervals in the case of *Cypridopsis* which had been for some time previously exposed to the sunlight. In these the positive condition, if found at all, was always of brief duration. (See p. 358.)

² This was most noticeably the case when the animals were brought from exposure to direct sunlight.

³ A similar phenomenon was observed by Loeb (*Archiv f. d. ges. Physiol.*, 1893, p. 96), in the case of *Copepods*, but he did not attempt to explain it.

checked the response altogether. Moreover, the change from negative to positive takes place always in the end of the trough in which there is no shadow.

Again, this change might be due to something in the character of the ends themselves. Wedges of cork were therefore substituted which also cut off the shadow, but they did not affect the results.



FIGURE 2.

And, finally, the same results were obtained when the animals were placed in a prism containing water and so tipped (Fig. 2) that the water made its own edge at *x*, in contact with the air and the bottom of the prism. Here too, a *Cypridopsis* could be driven up and down the sloping bottom by shifting the light, while it changed as before from positive to negative or the reverse if left alone.

That the phenomena were in no way due to differences in intensity of light, was proved by placing over the trough a prism with a glass bottom (Fig. 3) similar to that used by Strasburger¹ and Davenport,² and containing a weak solution of India ink in water. The animals were dropped

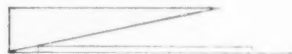


FIGURE 3.

into the trough and the light shifted from end to end as before, while the prism was not moved. The positive individuals moved always toward the source of light, going either to the lighter or darker end, as the case might be. If the light remained stationary for a time at either end, the characteristic change from positive to negative took place in some of the individuals. If the light was again shifted, the remaining positive animals followed the light as before, while the negative ones moved always away from it, into either the darker or lighter end. This made it clear that even considerable variations in the intensity of light at the two ends had no power to change the response.

Loeb's³ experiments with *Polygordius* larvæ and Copepods suggested that the change might be due to small variations in temperature. But readings taken at different places in the trough showed it to be uniform throughout.

Another possible explanation of the changes observed was that the position of the light above the plane of the trough and the angle at which the rays entered it might in some way affect the response. To

¹ STRASBURGER: *loc. cit.*, p. 35.

² DAVENPORT: *loc. cit.*, p. 204.

³ LOEB: *Archiv f. d. ges. Physiol.*, 1893, pp. 90-96.

avoid this possibility I used another trough exactly similar to the first, except that the ends were of glass. I then placed the light so that the rays entered the trough perpendicular to its ends, and parallel to the surface of the water inside. These experiments showed only that in some cases the time required for the change was longer, but even this was uncertain. It also seemed to me possible that if the trough were longer so that the animal would have to swim a greater distance before touching the end, it might change its course, and hence the character of its response, before the end was reached. To determine this I used a longer trough, $18 \times 3 \times 3$ inches, painted black inside, and with ends of glass like the smaller one. But in this the same results were obtained. One *Cypridopsis* would swim the entire length of the dish, showing no sign of turning, would reach the end and swim about there for more or less time, then suddenly turn and swim back. And in both cases it would respond, as before, to changes in the position of the light. In fact, this change of response seemed to be characteristic of the animal, for I met it under all circumstances, in all kinds of dishes, and in all intensities of light. There was a constant interchange of positive and negative individuals, each one, for the time being, perfectly definite in its reaction, and ready to follow any shifting in the position of the light. And it was this characteristic that had made my earlier results so irregular and inexplicable, and which could have been discovered only by observations of isolated individuals.

Davenport¹ in his experiments with *Daphnia*, dropped several individuals at once into a trough, and after a given time, let down a series of partitions which divided the trough into three parts. He then counted the number of individuals in each of the cells so formed, and found that the greater number of *Daphnia* had moved toward the light. That the usual response of *Daphnia* is a positive one, there can be no doubt, and yet I have found while working with *Cypridopsis*, individual *Daphnia* as markedly negative as the majority were positive; and the question arises whether they too might not, under certain conditions, show a similar change of character. It is evident that had Davenport's method been followed in my earlier set of experiments, and a time limit of sixty seconds been used, the results would have pointed strongly to *Cypridopsis* as a positive form; while later experiments show that with a longer limit, exactly opposite conclusions might have been reached.

¹ DAVENPORT, C. B., and CANNON, W. B.: *Journal of Physiology*, 1897, xxi, p. 26.

I have found in the literature of the subject only two mentions of similar phenomena. The first was made by Strasburger¹ with reference to *Ulothrix* swarm spores. He says:² "On the positive as well as on the negative side individual swarm-spores may constantly be observed to leave the margin suddenly and hasten straight through the drops to the other margin. This exchange between the margins takes place uninterruptedly." This behavior he explains later, saying that the spores were then probably exposed to nearly their optimum intensity, and that therefore it was indifferent to them whether they moved toward or from the light, so long as their path lay parallel to the rays. That this explanation does not apply to *Cypridopsis* appears from the fact that the same phenomenon is met with in a wide range of intensities, not only of gaslight but also³ of diffuse light and direct sunlight.

The second observation of this kind was made by Groom and Loeb⁴ on *Nauplii*. After exposure to light they become negative, but if covered for a few moments and then again exposed, they are temporarily positive. Loeb maintains that the character of the response is modified by the preceding intensity to which the organism has just been exposed. But it is hard to believe that in the case of *Cypridopsis* a short exposure to the intensity of light at the positive end makes it negative, and a similar exposure at the negative end makes it positive. For (1) in the shorter trough with parallel light, the intensity can differ but little at the two ends; (2) the change takes place no sooner in the very long trough than in the short one; and (3) when the trough is covered with an ink prism the same changes take place *at either end*.⁵

The problem, therefore, which presents itself is as follows: *Cypri-*

¹ STRASBURGER: *loc. cit.*, p. 17.

² "Ununterbrochen bemerkt man auch, sowohl an der positiven als auch an der negativen Seite, einzelne Schwärmer, die plötzlich den Rand verlassen und gerade aus durch den Tropfen nach dem anderen Rande eilen. Ein solcher Austausch findet ununterbrochen zwischen beiden Rändern statt."

³ See pp. 359 and 363.

⁴ GROOM, T. T., and LOEB, J.: *Biologisches Centralblatt*, 1890, x, pp. 171-2.

⁵ It is worth noting in this connection that in the spring of 1898, while experimenting on some small *Phyllopod*s, I observed a somewhat similar phenomenon. When the organisms were first taken from the aquarium to a glass evaporating dish and set before the window, they gathered in swarms on the negative side of the dish, but if left undisturbed for some time they moved over to the positive side. If the dish was again shaken, they again swarmed on the negative side, to return as before when left for a time. No explanation for this behavior offered itself.

dopsis vidua, when exposed to light, undergoes successive changes in response from positive to negative, from negative to positive. The possible factors determining these changes may be (1) internal, (2) external, or (3) both working together. Of the external factors, variations in light intensity and in temperature may be eliminated. The one remaining which suggests itself most obviously is contact, and my first conclusion was that this was the cause of all the phenomena. This view was strengthened by the following experiment.

A maze of seventy-five very fine sewing needles (No. 12), was arranged in the middle of the small trough, and it was placed in front of the light as before. A *Cypridopsis* was dropped into the farther end, and its course watched. As the needles were set very close together, the animal in its course toward the light had to thread its way among them, and came into forcible contact with a number in succession. In the majority of cases, the response of such an individual changed from positive to negative in its first course through the needles, and it often turned before it had found the end of the maze. A few animals wandered for some time among the needles without finding their way out, and then usually came out from the end which they had entered, being then negative in response. In these experiments also, the positive individuals changed more quickly to negative than did the negative to positive. That contact may play a part in the transformation was thus made clear. But upon more careful consideration I saw that it failed to explain all the results satisfactorily, and in October and November, 1899, I made a somewhat closer study of the problem.

In the first place it was evident that the term "contact" must be used in a broad sense to cover not only the forcible hitting of the animal against the ends of the dish, but also the mechanical stimulus which it receives when picked up or shaken in the water. But this latter stimulus acts always to change a negative animal to positive, never a positive one to negative, while contact with the end apparently effects both changes. Have we therefore two entirely different factors at work here? Or is one but a more or less intense form of the other? Or, finally, does some third element enter in? If contact is the sole cause why should the positive reaction be changed to negative sooner than the negative to positive? And again, why should the same individual change with greater ease at one time than at another? I found that an animal that had just become negative, and had therefore just left the positive end, was generally more

easily changed to positive than one which had progressed for some distance down the trough toward the negative end.

It seemed to me, therefore, that the important point remaining to be determined was whether the change in response could take place independently of external stimuli; or, in other words, whether the change was due entirely to the working of external factors or might result wholly from internal causes. For this purpose I used the larger trough, dropping the animals in as before. My purpose was by constantly changing the position of the light, to keep the *Cypridopsis* from touching either end at any time; and to see whether in this case, contact being eliminated, the usual change in response would occur. In nearly all cases the result was as follows: Each animal when first dropped in was positive; by shifting the light I was able to keep it so for varying lengths of time—dependent to a certain extent upon the intensity to which it had been exposed previous to the experiment (*cf.* pp. 353 and 363). Sooner or later, the average time being from two to five minutes, the positive response ceased and was followed sometimes by a period of torpidity, due possibly to fatigue, in which no change in the light produced any effect whatever. After this torpid period or without it, the negative response began. And in all cases this persisted, as long as I continued to shift the light, or until the animal was evidently fatigued. One individual I kept negative for half an hour in this way. But I was able at any time, by more or less agitation of the water or by picking up the *Cypridopsis*, to induce a temporary positive response.

In order to assure myself of the truth of these results I repeated the experiments in a trough 8 feet long and 7 inches wide. In this the light needed to be moved much less often, and so possible confusion was avoided. *In all cases the positive response was temporary,¹ while the negative one persisted as long as the animal could be kept in motion.* In one case I held the light over the middle of the trough. The animal would stop and swim about under it for more or less time and then turn and swim back as it had come.

These final experiments make it clear, I think, that the normal response to light of *Cypridopsis vidua* is not, as would at first appear, positive. The positive condition is, on the other hand, only a temporary one, induced by some mechanical stimulus, such as contact, jarring, etc., and sooner or later, according to the intensity of

¹ The duration of the positive response was shorter when the *Cypridopsis* had been previously exposed to direct sunlight.

light to which the organism has been for some time exposed, it gives place to the more stable negative condition.

Positive and negative responses, then, represent different phases in the internal condition of the animal. Positive changes to negative, independently of external conditions, after the lapse of a varying length of time; negative gives place to positive much less readily, and only when induced to change by the action of some mechanical stimulus. In the latter external factors are the more immediate cause, while in the former only internal conditions operate.

II. EXPERIMENTS IN DIFFUSE DAYLIGHT.

These experiments were made in order to ascertain whether the phenomena described before were to be met with only in artificial light (much weaker than that normal to the individuals), or whether the reactions were also characteristic of the form under normal conditions. For this purpose the smaller trough was placed close to a window where it received only diffuse light. All light except that coming directly from the window was cut off by means of black triangular screens. The bottom of the trough was marked into thirds, and four or five animals were dropped into the middle third. A series of observations was then taken, every half minute or every minute, of the number of individuals in each third. The results when averaged were as follows. Only the first set of observations and one after from eight to ten minutes are given: —

Interval in minutes.	+ Third.	* Middle.	— Third.	Total	Remarks
0.5-1.0	82 = 59%	39 = 28%	18 = 13%	139	
8-10	31 = 22.6%	30 = 21.9%	76 = 55.5%	137	2 lost.

From this table it will be seen that the first response in the majority of cases was positive, and that later there followed an accumulation of individuals in the negative end. If the experiment was continued longer, there was a constant interchange between the two ends, but the greater number of animals were found in the negative end. If an evaporating dish was left in the window for some time, properly screened from the room about, the majority collected on the negative side, but there was always a certain amount of interchange as above.

III. EXPERIMENTS IN DIRECT SUNLIGHT.

This series of experiments has no direct connection with those given above, but was suggested by Oltmanns' paper¹ of 1892. Oltmanns worked with a large number of plant forms, motile and others, *Volvox globator*, and *V. minor*, *Spirogyra*, *Mesocarpus*, *Vaucheria*, etc. The experiments on *Volvox* are the ones to which I wish to direct especial attention.

Oltmanns used a box, two opposite sides of which consisted of prisms of gelatine containing India ink. The dish containing *Volvox*

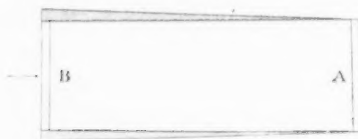


FIGURE 4.

was put under this box and the latter placed before a window as in Fig. 4, so that the sunlight fell on one end at an upward angle but parallel to the sides. The brightest region was thus at A, the darkest at B; but, he argues, the regions of equal

intensity lay, not in planes vertical to the surface of the water, but at more or less of an angle with it toward the position of the sun.² Under these conditions he found that the parthenogenetic individuals collected in great numbers in the brightest part, while the females arranged themselves in rows which were longer at the darker end, at a depth corresponding, as he believed, to their optimum intensity. He then placed the box with the sun shining on the prism from an elevation of 45° but parallel to the closed ends. Under these conditions all left the brightest region, the females gathering in the darkest, and the parthenogenetic individuals in a somewhat lighter part. If a cloud covered the sun, all moved further toward the clearer end. In no instance, he says, was there any sign of movement in the direction of the light rays. From these and other experiments Oltmanns concludes that every organism is attuned to a certain intensity of light, and that all its responses to light stimuli result from attempts to reach this optimum. The influence of ray direction is only secondary. An organism may move toward the source of light and parallel to its rays, but it does so because, by so doing, it is approaching more nearly the intensity which is its optimum. But Olt-

¹ OLTMANNS, F.: *Flora*, 1892, lxxv, pp. 183-266.

² The argument is not at all clear, but this is, I think, what is meant.

manns' experiments on *Volvox* were inadequate to prove his point. For in the first experiment, in which the sun's rays fell at an angle on a closed end of the trough, and parallel to the sides, only diffuse light of uncertain direction could penetrate to the interior, and therefore the exact position of planes of equal intensity could not be accurately determined. In the second experiment, the angle at which the direct rays entered, made accurate results less possible; but apart from this, as my experiments will show, the conditions were not as simple as they seemed.

It was my purpose, therefore, to modify Oltmanns' method in such a way as to avoid possible objections and to determine whether such forms as *Cypridopsis* or *Daphnia* would evince any preference for a definite light intensity, or whether the direction of movement might not depend solely on the direction of the rays of light.

The apparatus used was a tin box $12 \times 9 \times 5$ inches, with one end of glass and a close cover. The entire interior, excepting the glass plate, was painted black. Over the glass end I used an India ink prism, varying the thickness and intensity at different times. On this prism the sunlight was reflected by means of a mirror, so that the rays entered either perpendicular to the prism or at an angle with it, as desired, but always parallel to the surface of the water within.¹ If the sunlight fall perpendicular to the prism, in theory there should be in the water parallel planes of light of regularly decreasing intensity, corresponding to the gradation in the prism (AA' to BB' , Fig. 5), that is, no light should reach A' except that which has passed through A , or B' except that which has passed through B . But that this is not the case may be easily seen, for there are of necessity innumerable particles in the water which will diffuse light, for example, from B to A' .² The conditions are therefore not so simple as they at first appear. An



FIGURE 5.

¹ In the later experiments a glass plate was placed in the water at about half its depth, and the animals were dropped on this, so that any shadow from the front edge of the trough falling on the bottom might be avoided, and also that the diffuse rays from above might be balanced by similar ones from below.

² This can be demonstrated by making observations through an aquarium of which $A'B'$ is also of glass. The light from B is very distinct and an important factor in the illumination of A' .

organism placed at A' may be influenced by (1) the *direct* light which passes through A, and (2) the *diffuse* light coming from B. The relative value of these two forces with respect to the organism will therefore determine their resultant effect, and hence the direction of its response. If a Cypridopsis or Daphnia be dropped into the trough at A' and be seen to move in a line which forms a distinct angle with AA', and toward B, nothing can be inferred in regard to its response to light *intensity*, but we may only conclude that the light from B, as well as that from A, is exerting a sensible effect upon its movement. Even this diagonal course, therefore, may be determined by the *direction* of the rays impinging upon the organism. This theory was, I found, confirmed with large numbers of Cypridopsis on which I worked, and even more distinctly with Daphnia, which are more sensitive to slight variations in the direction of the incoming rays. Individuals were brought from diffuse daylight, in which, as has been seen, their first response is positive, and placed for a few minutes in the black dish used in Experiment I. This treatment made their subsequent response somewhat more sensitive. They were then dropped at various points into the box. In the majority of cases a *distinctly diagonal course was taken*. If a black screen was placed in front of the lighter end of the prism, so cutting off the strong diffuse rays, the path taken became parallel to the (stronger) direct rays; on removing the screen the diagonal course was resumed. In the case of some Daphnia this operation could be repeated several times as they moved down the box, their direction changing very quickly with changes in the light. The entire course of one may be represented by the line in Fig. 5. With a Cypridopsis only one or two such changes could be effected in one trip from end to end.

To prove that the light from B was the factor which caused these changes in direction, the screen was interposed over the darker end of the prism A, thus cutting off from the organism all direct rays; a still more distinctly diagonal course resulted. If a Cypridopsis or Daphnia was dropped in at B' and B covered, a diagonal movement toward A followed, but if B remained uncovered, the course taken was parallel to the direct rays, the very weak diffuse rays from A having no appreciable effect in altering the course of the animal.

If the conclusions given above be correct, it should be possible to cause Cypridopsis or Daphnia to move perpendicular to the lighted end of the trough by reflecting the sun's rays into the trough at an angle with the prism, as shown in Fig. 6, for at a certain angle the

resultant movement of the two forces should be in the direction AA' . In this case, part of the box is in shadow and care must be taken to drop the animals outside of this and within the influence of the direct rays. *The anticipated result was obtained with both Cypridopsis and Daphnia.* At a certain¹ angle of the direct rays with AB the individual moved in a line parallel to the sides of the trough; if the angle was increased a corresponding bending of the course toward A was noticeable.² (For these more delicate reactions *Daphnia* were found more satisfactory, for the angle of the rays had to be much greater to induce the *Cypridopsis* to move into a region of less intensity.) If either a *Cypridopsis* or *Daphnia* was dropped into the part of the dish which was in shadow, *i.e.*, into which no direct rays found their way, it moved diagonally toward B until it came under the influence of the direct rays, when its course was altered as above stated.



FIGURE 6.

Finally, I believed that the experiments would be complete if the reactions of a negative *Cypridopsis* could be observed, as its response should be the exact opposite of that of a positive individual. But there is the difficulty that when the organisms are brought from diffuse light, or from the black dish, into the trough, they are made distinctly positive by the process of transfer; and those which are left in the trough gradually collect in the light region, moving parallel to the side from the positive to negative ends in characteristic manner. Neither of these groups of individuals is available therefore for the purpose. But I found that after long exposure to direct sunlight *Cypridopsis* became so strongly negative that it was very difficult to change it by any amount of disturbance. It proved to be strongly negative when brought into gaslight, also in diffuse light and in sunlight that had passed through the prism. Such negative individuals when dropped into the box at B , responded as expected, moving in a diagonal course toward A' , if the light was perpendicular to AB , and parallel to BB' when the light was at an angle toward A .

¹ Dependent on the intensity of the light and the thickness of the prism.

² In the dark room experiments in which the prism was used, the direct rays formed such a great angle with the diffuse that the influence of the latter did not appear.

Dropped within the edge of the shadow a negative Cypridopsis moved away from the strongest light, namely, in the direction BA'; while a positive Daphnia dropped in at the same time, moved in an exactly opposite direction.

Davenport,¹ in his "Experimental Morphology," makes the provisional statement that "if two rays of different intensities making an angle with each other, fall upon the organism, it apparently moves in the direction of the intenser ray, if free to do so." That this is not the case in this form at least, has been seen. Each ray exerts its influence independently of the other, and the direction of the resultant movement of the organism is conditioned by the direction and the relative intensity of the impinging rays.

In the November number of this Journal, there appeared an article by R. M. Yerkes on the "Reaction of Entomostraca to Stimulation by Light." In this the author endeavors to prove that the organism *Simocephalus vetulus* Mueller may give a photopathic reaction as well as the phototactic one which Davenport and Cannon proved for it in 1897. For this purpose he used an apparatus which was similar in principle to that used in my third series of experiments, except that the trough was proportionately much narrower, thus preventing, as he claims, all possibility of phototactic reaction. The light, first diffused, passed through an ink prism into the side of the trough in a direction perpendicular to its long axis. The trough was divided into compartments and in them certain individuals were placed. The partitions were raised after a certain time, three minutes being most favorable to the desired result. Yerkes found that at the end of this time the majority of individuals had gathered in the positive (lighter) end of the trough. He concludes therefore that "*Simocephalus vetulus* Mueller is positively (+) photopathic." This is, I believe, another instance of an erroneous conclusion being drawn from the results of experiments with a prism. To an individual *Simocephalus* placed in the darker part of the trough, the source of light is the lighter end of the trough, and toward this it moves. The "source of light" is, it seems to me, only another name for the point or region from which the most intense rays come; and when an organism, placed in the conditions created by Yerkes, moves toward the lighter region, it is only moving in the path of the most intense rays that strike it, and is then still phototactic.

¹ DAVENPORT: *loc. cit.*, p. 208.

Another point to which I should like to call attention is the interesting variation caused by changing the time of the experiment. Three minutes gave the maximum positive response (and was therefore chosen as the most favorable period), while "when the time became longer than three minutes, the result showed a decrease." "It seems probable," says the author, "that during the first two minutes all the animals have time to move to the most intensely lighted portion of the trough, *i. e.*, space 1. After moving about for a few seconds there they begin to move in the only direction possible, namely, toward the negative end of the trough. Whether this is due to a gradual lessening of the stimulation is uncertain. It might well be that after a few trips back and forth most of the animals would come to rest in that intensity of light most suitable for them,—that to which they are 'attuned.'" This view is not maintained by experimental evidence and seems to me improbable. I should like to suggest that the true explanation may be found in some such phenomenon as that which I have described in the case of *Cypridopsis vidua*.

SUMMARY.

I. The condition of an individual of the species *Cypridopsis vidua*, with reference to its reaction to light, changes, under constant conditions of temperature and light intensity, from positive to negative, from negative to positive. The former change is brought about solely by internal causes, while the more immediate cause of the latter is an external one, namely, mechanical stimulation.

II. The direction of movement of *Cypridopsis* and of *Daphnia* in response to light does not result from an effort on the part of the individual to reach a certain optimum intensity. It is determined (1) by the directions of all the impinging rays, and (2) by the relative value of these rays as forces acting upon the organism, *i. e.*, by their relative intensities. The resultant direction could be found by compounding all these forces if their direction and relative value were known.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE CALIFORNIA HAGFISH, *POLISTOTREMA STOUTI*.¹ —I. THE ANATOMY AND PHYSIOLOGY OF THE CAUDAL HEART.

BY CHARLES WILSON GREENE.

[From the Physiological Laboratory of the Leland Stanford Junior University.]

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IN the classical anatomical researches of Johannes Müller² on the circulatory system of the hagfish, *Bdellostoma forsteri*, there is no reference to a caudal heart. However, the great anatomist was

¹ Gill (Proceedings of the United States National Museum, 1882, v, pp. 517-520) divides the genus *Heptatrema* Duméril, 1829 (or earlier), *Bdellostoma* Müller, 1834, into two genera. The generic distinction is based on the number of pairs of branchiæ. The species with ten or eleven pairs of gills are placed in a new genus, those with seven (or six) pairs remain as *Heptatrema* Duméril, which, according to Gill, takes priority over *Bdellostoma* Müller. For the new genus Gill proposes the name *Polistotrema*. By this arrangement the name *Bdellostoma* which has become classical in morphological literature by the work of Johannes Müller himself and of numerous later investigators is abandoned, or at most restricted to the significance of a common name.

The scientific name of the hagfish I have used in these experiments is *Polistotrema stouti*. The animal was originally described by Lockington under the name *Bdellostoma stouti*.

² MÜLLER, J.: Vergleichende Anatomie der Myxinoïden, Dritte Fortsetzung über das Gefässsystem. Abhandlungen der königl. Akademie der Wissenschaften aus den Jahren 1839-1841, Berlin, 1841.

well acquainted with the fact that the eel possesses such an organ.¹ The presence of a caudal heart in the Myxinoids was first observed by Gustav Retzius² in the tail of *Myxine glutinosa*. While investigating other things, Retzius' attention was quite accidentally directed to a paired pulsating organ in the tail of this animal. The observations he made, though brief, are fundamental. He says that in *Myxine*, "The pulsating organ in medium sized animals lies with its posterior end about one centimetre from the tip of the tail, and extends forward for about a half centimetre. It lies under the chorda dorsalis, is egg-shaped, and has a length of about five millimetres and a width of two to two and a half millimetres. In a surface view it appears to be flattened, although it becomes thicker at every relaxation and filling." Retzius figures a transverse section through the caudal heart of *Myxine* and says: "There are present two parallel and somewhat flattened sacs which are separated from each other by a median vertical cartilaginous plate. The paired sacs are surrounded by a fibrous connective tissue wall in which no muscle fibres are visible. But there lies on the outer side of each sac a thin lamella of transversely striated muscle fibres." Retzius further describes these muscle fibres as extending downward and forward to an insertion "on the knob of the median cartilaginous plate," and adds that they "appear to exist for the purpose of setting this plate in motion."

In describing the physiological activity of this organ Retzius says: "At each systole the knob of the cartilaginous plate turns from the left toward the right, and in this movement the two paired sacs empty their contents into the caudal vein. Then the knob turns again toward the left and remains quiet a moment. Then comes a new movement toward the right with an emptying of the sacs, etc. The two flat muscles on the sides are obviously the motors which press at the same time (*gleichzeitig*) upon the walls of the heart sacs and force their contents forward. The two muscles are evidently specialized for this apparatus and should therefore in the future be called *Musculi*

¹ MÜLLER, J.: Müller's Archiv, 1842, p. 477. See also JONES: Philosophical Transactions, 1868. In this paper Jones refers to the discovery of the caudal heart of the eel by Marshall Hall in 1831, and to a discussion of the subject by J. Müller in Poggendorf's Annalen for 1832. Judging from Jones's description the activity of the caudal heart of the eel is so different from that in the hagfish that a comparison is scarcely possible.

² RETZIUS, G.: Biologische Untersuchungen, Neue Folge, 1890, i, pp. 94-96.

cordis caudalis."¹ Retzius could not force blood from the caudal vein back into the caudal heart, and finding that an injection fluid passed into the vein went only to the beginning of the sac he concluded that "there is present here a kind of valvular apparatus which prevents the return of the blood into the sac."

Retzius did not discover an opening into the caudal heart of *Myxine* but cites A. Klinckowström² as having found in the caudal vein an injection mass which he had introduced into the subcutaneous lymph spaces. In a later paper before the Biological Society Klinckowström³ gives the details of the anatomical relations between the subcutaneous lymph sacs and the caudal heart in *Myxine glutinosa* as follows:

"In the caudal region the subcutaneous lymph sacs give off a series of metamERICALLY arranged vessels which follow the rays of the caudal cartilage. These paired vessels anastomose partly with each other and partly also with a paired vessel which runs along the border of the caudal cartilage and empties into the paired caudal heart discovered by Retzius."⁴

ANATOMY OF THE CAUDAL HEART OF THE HAGFISH.

The caudal heart in *Polistotrema* is apparently quite similar in location and in general features to that found by Retzius in *Myxine glutinosa*. The contractions of its muscles can be observed in the living animal as rhythmic movements of the skin at a point about two centimetres from the end of the tail.

¹ "Bei jeder Systole kehrte sich der Knopf der Knorpelplatte von links nach rechts, und dabei entleerten die paarigen Herzsäcke ihren Inhalt in die Caudalvene. Dann kehrte die Knopf wieder nach links zurück und blieb einen Moment stille; so kam eine neue Verschiebung nach rechts mit einer Ausleerung der Säcke u. s. w. Dabei waren deutlich die beiden seitlichen platten Muskeln die Motoren, welche gleichzeitig auf die Wände der Herzsäcke drücken und den Inhalt vorwärts verschieben. Die beiden Muskeln sind offenbar für diesen Apparat bestimmt und sollen deshalb bis auf weiteres *Musculi cordis caudalis* genannt werden."

² KLINCKOWSTRÖM, A.: Verhandlungen des biologischen Vereins in Stockholm, 1890, ii, No. 6, quoted by Retzius.

³ KLINCKOWSTRÖM, A.: *ibid.*, 1891, iv, Nos. 1 and 2.

⁴ "Am caudalen Theil geben die subcutanen Lymphsäcke eine Serie metamer-angeordneter Gefässe ab, die den Strahlen der Caudalknorpel folgen. Diese paarigen Gefässe anastomosiren theils unter einander theils auch mit einen paarigen, am Rande des Caudalknorpels verlaufenden Gefässes, das in das paarige, von Retzius entdeckte Caudalherz einmündet."

The exact location of the caudal heart may be seen by consulting Fig. 1. It is a bilaterally symmetrical organ consisting of two sacs, located one on each side of a median caudal cartilage and under the chorda dorsalis as in *Myxine*. In an individual forty-five centimetres long the middle point of each sac lay two centimetres from the tip of the tail. Each cavity of the heart in this specimen was flattened in the transverse diameter and, viewed from the side, was somewhat egg-shaped in outline with its widest portion directed anteriorly. At the widest point it measured 2.8 mm., while it was 0.3 mm. in length.

Each cavity of the caudal heart is connected with the caudal vein by a short tube located at the anterior and superior margin of the organ. This small tube or vein runs anteriorly and joins its fellow of the opposite side immediately in front of the anterior border of the vertical cartilage, the two vessels forming the beginning of the caudal vein. The vessel on the right in the above individual is 1.5 mm. long, by 0.4-0.5 mm. in diameter. In other specimens this vessel was shorter, the caudal heart seeming in fact to open almost directly into the caudal vein. This connecting vessel possesses a pair of very delicate membranous pouches or valves of the semilunar type. The valves open anteriorly, allowing the blood to flow into the caudal vein. The slit between them extends dorso-ventrally.

In its anterior ventral wall each sac of the caudal heart possesses a free opening or ostium which brings it into free communication with the adjoining sinus described below. In the above individual the ostium is oval and of about the same diameter as the outflow vessel. In other specimens this opening forms a longitudinal slit. This opening also is guarded by valves. Around the inner border of the opening there is a very delicate raised membrane so placed as to form

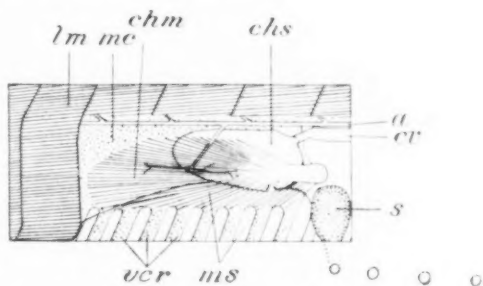


FIGURE 1. The caudal heart of the hagfish. The figure represents the tail in outline, the skin and lateral muscles having been removed from directly over the heart. Magnified three diameters. *a*, artery; *chm*, caudal heart muscle; *chs*, caudal heart sac; *cv*, caudal vein; *lm*, lateral muscle; *mc*, median cartilage; *ms*, median sinus; *s*, slime gland; *ucr*, ventral caudal rays.

two opposing longitudinal flaps which have somewhat thickened free edges. Anteriorly the free border of this membrane is attached to the cartilaginous knob. Posteriorly, in some cases at least, there is an attachment to the inner surface of the heart wall. The valve thus takes the form of a longitudinal slit which offers no resistance to the flow of liquid through the opening into the heart, but which effectually blocks the outflow.

In *Polistotrema* the subcutaneous lymph sacs are connected by an open system of channels with the caudal heart, as Klinckowstrom demonstrated for *Myxine*. As is well known, the skin of the hagfish is joined to the body dorsally and ventrally by numerous strands instead of by a continuous layer of subcutaneous connective tissue; thus a network of spaces or channels is formed. In the median ventral line in the caudal region, and around and between the cartilaginous fin rays, channels penetrate between the lateral muscles and unite in a fairly well-marked median space or sinus. This sinus is located around the bases of the ventral caudal fin rays and around the ventral border and sides of the median cartilage. Its roof is formed in part on either side of the plate by the floors of the caudal heart sacs. The antero-ventral opening of each sac connects this sinus directly with the cavity of the heart. Although the caudal sinus is partially divided by the caudal cartilage, still the two sides are in wide open connection through the spaces between the fin rays.

The muscles of the caudal heart, the *musculi cordis caudalis*, one for each side of the body, are situated on the external wall of each of the two cavities. They form no organic part of the heart sacs themselves, since the muscles are readily separated from the wall of the sacs by dissection under a simple microscope. The muscles are attached anteriorly to a knob on the end of the median cartilaginous plate as in *Myxine*, and the fibres of each radiate in a general caudal direction to a broad attachment over the posterior lateral surface of the same cartilage. The dorsal fibres extend backward and upward, the median ones backward, while the ventral ones extend backward and downward and are attached along the bases of the cartilaginous fin rays of the region directly below the caudal heart. It is to be noted that this ventral portion of the muscle is not mentioned by Retzius in his discussion of the muscles in *Myxine*. This is a rather striking difference in the two forms.

The muscle on the right side in the individual mentioned above was 12 mm. long by 3 mm. wide at its broadest part. The anterior

end of the muscle, *i.e.*, the cartilaginous knob to which the muscle is attached, was 25 mm. from the tip of the tail, and was located directly behind the last mucous gland of the lateral series (Fig. 1.). A small artery distributes branches among the fibres of the muscle.

It follows from this anatomical arrangement that the two sacs of the caudal heart evidently receive liquid from a common source, the median sinus, and transfer the liquid into a common channel, the caudal vein. The interesting manner in which this anatomical mechanism accomplishes its function will be discussed in the physiological section which follows.

PHYSIOLOGY OF THE CAUDAL HEART IN THE HAGFISH.

Function of the caudal heart and its normal cycle. — In my dissections and experiments I invariably found a considerable amount of blood in the subcutaneous sacs in the hagfish. Since these animals are caught on hooks it would be impossible to assert whether or not ruptures occur in the blood vessels. If ruptures occur then transfusion into the subcutaneous spaces will of course take place. Granting, however, that the amount of blood in the subcutaneous spaces may sometimes be increased because of artificial lesions, still the presence of the blood is so universal that there is little doubt that it is a normal condition in the hagfish. In one large specimen selected at random the blood drawn from the subcutaneous spaces measured ten and eight-tenths cubic centimetres.

Whenever the lateral muscle is removed from over the caudal heart sac, there is always a flow of blood, due evidently to the rupture of the median sinus. There seems to be no question, therefore, that *the function of the caudal heart in the hagfish is to drive the blood of the subcutaneous spaces back into the circulatory system*, a conclusion arrived at from less direct evidence by Retzius in his study of *Myxine*. But the action of the organ is quite different in *Polistotrema* from that in *Myxine* as described by Retzius.

The caudal heart, as already indicated, is in reality only a pair of thin-walled sacs uniting the subcutaneous spaces with the caudal vein. The sacs are provided with valves and each is located between a plate of cartilage and a flat muscle; but it must be remembered, as stated above, that this muscle can scarcely be considered as forming a part of the wall of the sac itself, certainly not in any way comparable to the arrangement of the muscular tissue in the ventricle.

Nevertheless, these sacs are the means of propelling blood, and it is no travesty to call the structure a heart. But the manner in which the blood is propelled is radically different from that in which the ventricle accomplishes the same result. *The caudal heart sacs are filled and emptied by the contractions of the muscoli cordis caudalis, the muscles acting directly upon the median plate of cartilage.*

The movements resulting from the contractions of the heart muscles may be easily observed in the tail of the quiet animal as a rise and fall of the skin directly over the caudal heart. When observing this pulsating movement in the animal in the aquarium, one is at once impressed with the fact that there is a positive sinking as well as a pushing out of the skin of the tail on the side observed. This phenomenon is due to the fact that the two caudal heart muscles do not contract together, and, as in *Myxine*, "At every systole the two paired heart-sacs empty their contents in the caudal vein," but they contract alternately, emptying one side at a time. In the tracing given in Figure 2 the positive up stroke is produced by the

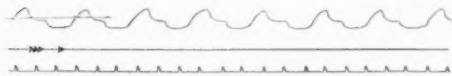


FIGURE 2. One half the original size. Experiment August 17, 1899. Showing the rhythmic alternate contractions of the caudal heart muscles of the hagfish *Polistotrema stouti*. Tracing taken by means of a heart lever resting directly on the muscle of one side. The stroke above the introduced base line is produced by the uppermost muscle, the stroke below by the opposite muscle. The time-marker records seconds.

contraction of the muscle upon which the heart lever rests, and the down stroke by the opposite muscle. When a muscle of either side contracts, for example, the right, it bends the ends of the median plate toward that side, cupping the cartilage quite decidedly.

The attachment of the muscle to the knob gives a strong leverage upon the anterior end of the plate, and this end is much more sharply bent than other portions. The result of the movement is the enlargement of the space between the contracting muscle and the cartilage, and the heart sac located in this space, Fig. 3, becomes filled with liquid by the movement, *i. e., the contraction of the cordis caudalis muscle on one side results in the filling of the caudal heart sac on the same side.*

The effect of the bending of the anterior end of the cartilage toward the right is to put the opposite muscle, the left, under strong tension around the convex surface of the cartilage on that side. By

this movement the left heart sac between its muscle and the cartilage is subjected to an increased external pressure which drives its liquid contents out into the caudal vein, *i. e.*, the contraction of the muscle on one side results in the emptying of the caudal heart sac on the opposite side. These facts have been repeatedly verified by direct observation upon specimens with the lateral muscles carefully removed from over the heart. With a simple microscope the flow of blood into and out of the heart can be determined with ease and certainty.

By reference to Fig. 1 it will be seen that the caudal heart muscles extend over the sides of the median sinus. The rhythmic application of pressure over this part of the sinus facilitates the filling of the sac lying immediately above.

It is to be remembered, as stated in the anatomical section above, that the anterior folds of the membranous valves guarding the openings into the caudal heart are attached to the cartilaginous knob. The movement of the knob to the right and to the left, therefore, must produce alternate tension and slackening of this valve,—tension when this muscle is passively stretched by the contractions of the opposite muscle, which would tend to close the valve; slackening when the muscle of the same side contracts, which would tend to open the valve. From the physiological and anatomical data given above it will be seen that *the contraction of the cordis caudalis muscle on one side opens the valve and fills the caudal heart sac on the same side, and at the same time closes the valve and empties the sac on the opposite side.*

The action of the caudal heart in the hagfish is thus directly comparable to the action of a double force-pump in the mechanical principles involved. Each alternate stroke produces two results, the filling of one chamber and the emptying of another at the same time. This as a type of pumping mechanism in the animal kingdom is

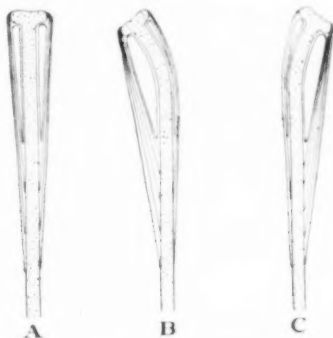


FIGURE 3. Diagram representing a horizontal section through the caudal heart sacs, the muscles, and the median cartilage. Magnified 3 diameters. *A*, position during rest; *B*, position during the contraction of the left muscle; *C*, position during the contraction of the right muscle.



FIGURE 4. About one half the original size. Tracing showing the periodic groups of contractions of the caudal heart muscles in the hagfish. Record taken by means of a heart lever resting over one of the heart muscles of the uninjured animal. The upper muscle produces an up stroke and the lower a down stroke. The time record is in quarter minutes.

unique and peculiar to this group of animals in so far as I can discover in the literature of the subject at my command.

The alternate movement of the cartilaginous plate to the right and to the left carries with it movements of the fin rays, a fact easily observed in the living uninjured animal, or better in the animal in which the lateral muscle has been removed; and it is probable that this movement facilitates the passage of the blood through the network of channels which lead from the subcutaneous sacs to the median caudal sinus.

The normal rhythm and its variations.—Observations upon animals resting quietly show that the normal rhythm consists in regularly repeated alternate contractions of the two caudal heart muscles, a fact mentioned above. The rate often remains fairly constant for relatively long periods of time. In the isolated tail, including the posterior nine or ten centimetres of the body, this constant regular rhythm may continue for an hour or more without interruption.

Yet in uninjured active animals one cannot but be impressed by the great irregularity of the rhythm. Whatever be the occasion of the irregularity, it invariably takes the form of a decrease in rate below that of the regular rhythm. Tracings taken by means of a counterpoised heart lever resting on the skin over the heart muscle exhibit the irregularities mentioned above. From a study of such tracings it is evident that the irregularities are due to variations in the rate of contraction of one muscle alone, or of both at the same time. It often happens that a single muscle may omit one or perhaps several contractions in succession. Sometimes one muscle may cease to contract altogether, while the other makes several contractions at a much slower and more irregular rate than usual.

Uninjured animals while lying perfectly quiet in the aquarium or on the experimental table may exhibit a regular rhythm for minutes at a time, but more often there are regularly recurring periods of active contractions and of perfect rest of these muscles, Fig. 4.

In such specimens, with the animals in a constant position, the alternate periods of rest and activity exhibit a regularity that is very striking, — a regularity indicative of some wave-like physiological changes which I am not at present prepared to explain. If the rate of the contractions in the periods of activity be observed directly or recorded it will be found constant for a few seconds; then becoming gradually slower for several seconds it finally ceases altogether. After a period of quiet the contractions begin again. In the new active period the rate rapidly increases up to the normal, remains quite constant for a time, then becomes slower and ceases as before. In one such period lasting one hundred and twenty seconds the rate reached the maximum in ten seconds, then slowly decreased until the end of the period, the rate changing from one contraction in one and five-sixths seconds to one in three and two-thirds seconds.

In the tracing given in Fig. 4 it will be seen that sometimes the sequence of the contractions of the muscles is not even interrupted by the long pause. It often happens that when a pause appears one muscle ceases to contract several seconds before the other. The rhythm may not be re-established in both muscles at the same time.

Experiment August 17, 1899. — Normal animal resting quietly on a horizontal board. The movements of the caudal heart muscles were recorded by means of a heart lever placed on the skin of the tail directly over the muscles. The muscles show alternate periods of rhythmic contractility and of perfect

TABLE I.

Experiment August 17, 1899. Showing fourteen alternate periods of rest and rhythmic activity in an uninjured animal. Numbers taken from a tracing. Where two numbers are given the first applies to the uppermost muscle, the one over which the lever rests, and the second number to the lower muscle.

Periods of Rest.			Periods of Activity.			Number of Contractions.
1	72	seconds.	2	120/113	seconds.	56.53
3	72/78	"	4	120	"	55.54
5	71.67	"	6	90.93	"	42.44
7	83	"	8	93	"	52
9	75	"	10	107	"	51
11	86	"	12	106	"	51
13	81	"	14	104	"	54

quiet as given in the accompanying table. In the first part of the experiment the muscles of the two sides did not give the same number of contractions. In one case, the second period, the uppermost muscle gave three contractions after the lower one ceased to act, and in the sixth period the lower muscle gave two contractions before the upper one began to contract. The last three periods of activity and the intermediate periods of rest are given in Fig. 4.

The relative duration of these periods of activity and rest in the caudal heart muscles is very decidedly influenced by the position of the body of the animal. The tracing figured was taken with the animal in the horizontal position. Observations upon animals with the head higher than the tail, or the reverse, are not numerous enough to allow of positive deductions. However, the indications, as the accompanying notes will illustrate, are that with the animal's tail lower than its head the activity of the caudal heart muscles is relatively increased and *vice versa*.

TABLE II.

Experiment August 17, 1899. Animal quiet, suspended by the tail.
Direct observation showed:

14	contractions, then quiet for 2 minutes, followed by
20	" " " " 2 " " " "
23	" " " " 1 " 58 seconds, followed by
22	" " " " 2 " 15 " " "
24	"

Animal reversed, *i. e.*, tail down:

55 consecutive contractions.

Animal in original position, *i. e.*, head down:

25 contractions, then quiet for 2 minutes, followed by

24 "

Animal changed to the horizontal position:

	Quiet for 2 minutes, 30 seconds, followed by
30	contractions, then " 2 minutes, followed by
20	" " " " 2 " " "
20	"

Animal with the tail down:

41 contractions up to the end of the experiment.

It must be remembered that when the animal's tail is lower than its head the mixed blood and lymph of the subcutaneous spaces runs down toward the tail and brings about pressure changes in that region. It is possible that this gives rise to sensory impulses which reflexly influence the activity of the caudal heart muscles.

Another striking and very significant phenomenon which must be

noticed here is the fact that whenever muscular movements of the body occur there is invariably an interference with the rhythm of the caudal heart muscles. Usually this interference amounts to a complete inhibition of the contractions. Sometimes when the body movements are slight the rhythm of the heart muscles becomes slower and more irregular but is not completely stopped.

General movements of the body result from a discharge of motor impulses from the central nervous system to the lateral muscles. But in the hagfish a discharge of motor impulses which produces contractions of the lateral muscles is associated with nervous activity which brings about cessation of motor action in the caudal heart muscles, a fact which at first sight seems anomalous and contradictory, but which may be satisfactorily explained after a discussion of the evidence of nervous regulation of the caudal heart muscles.

The relation of the nervous system to the contractions of the caudal heart muscles.—*The vagus without influence.*—I was unable to demonstrate any influence upon the rhythm of the caudal heart muscles following stimulation of the vagus nerve. In many experiments for other purposes where the caudal portion of the animal was cut away from the rest of the body no special influence was noticed upon the rate of contraction of the caudal heart muscles. It would seem, therefore, that the muscles in their rhythmic action are not influenced by fibres which pass by way of the tenth cranial nerve trunks.

The effect of peripheral stimulation of the spinal cord.—In demonstrating this point only the posterior part of the body was used. This part was pinned firmly to a frog-board in such a way as to reduce to a minimum any movements of the caudal cartilage that might result from contraction of the lateral muscles. The caudal heart

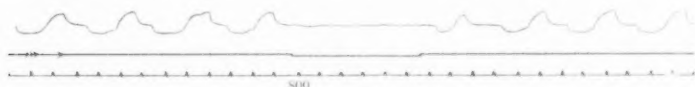


FIGURE 5. *Experiment August 17, 1899.* One half the original size. Inhibition of the caudal heart muscles of the hagfish produced by stimulating the peripheral end of the spinal cord for six seconds. Strength of stimulus 800 units (Petzold induction coil fed by one Edison-Lalande battery). The time-marker records seconds.

muscle of the upper side was then uncovered and a heart lever adjusted to it. A portion of the spinal cord was isolated, sectioned, and the caudal end stimulated with an interrupted current. Under these experimental conditions and with a moderate strength of the interrupted current, Fig. 5, *stimulation of the peripheral end of the*

spinal cord in the hagfish is invariably followed by inhibition of the contractions of the caudal heart muscles. In my experiments only the relative change in the intensity of the stimulating current as indicated by the position of the secondary coil with reference to the primary can be given. In the earlier experiments with the secondary coil eight centimetres from the primary total inhibition usually follows faradaic stimulation. At ten centimetres, as a rule, no change is produced in the rate of contraction. In later experiments using a Petzold coil this range is indicated more definitely by Fig. 6. A stimulus

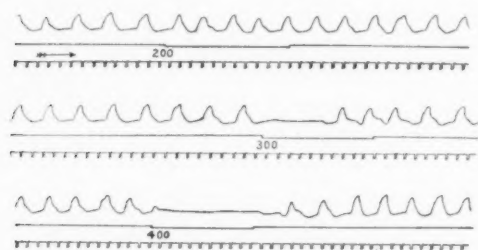


FIGURE 6. Experiment October 7, 1899. One half the original size. Consecutive stimulations of the peripheral end of the spinal cord of the hagfish with 200, 300, and 400 units of current (Petzold coil fed by one ammonium chloride element). Time record in seconds.

of moderate strength is followed by inhibition which begins and ends with the stimulus, Figs. 5 and 6. The exact latent period is difficult to determine. But the general experiments indicate, as do also the accompanying figures, that it is much shorter than the latent period of inhibition of the frog's heart upon vagus stimulation. The

promptness of the return of contractions upon cessation of the stimulus is always surprising. It is only with excessively strong stimuli or in fatigued specimens that inhibition lasts longer than the time of stimulation. With the secondary coil at four centimetres no after-inhibition followed; at two centimetres a partial after-inhibition occurred; with the secondary coil at zero centimetres the resulting total inhibition continued some time (six to eight seconds) after the stimulus ceased. An after slowing of the rate lasting for a few seconds occurred, following even moderate intensity of stimulation, but this slowing quickly passed away. The heart muscles usually took up their former rhythm immediately after stimulation ceased.

With a stimulation lasting for a relatively long time recovery from inhibition occurs, Fig. 7. This recovery may be only partial or it may be complete, as in Fig. 7. By gradually decreasing the strength of the stimulus an intensity is easily found which produces only slowing of the rhythmic contractions of the heart muscles. In some cases

this slowing is greater on one side than on the other. But in any given experiment the range of intensity of stimulus between that which produces no effect and that which gives total inhibition is exceedingly narrow.

It is interesting to note the relation of the activity of the two muscles of the heart as recorded by these experiments. In those cases where partial inhibition results the inhibition may be total on one side and not on the other, or it may be greater on one side than on the other. This implies that there is no absolute relation of the activity of the muscles of the two sides.

The same fact is indicated by other observations. For example, the contraction of the muscle of one side usually follows immediately upon that of the other side, with a slight pause before the second contraction of the first again. But there is no regularity as to which side initiates the alternate contractions. In a series of sixteen inhibitions where the lever rested directly on one of the muscles the number of cases in which the contractions were begun again by the exposed or upper muscle was exactly the same as the number initiated by the opposite muscle. In so far as this point is concerned it would seem that the fact that the muscle on one side was exposed to the air and the other not, made little difference. In fatigued examples, *i. e.*, in animals that have been under experiment for a long time, it may happen that the contractions become intermittent or entirely cease on one side while they go on uninterruptedly on the other side. If the muscle of the exposed side of the caudal heart be destroyed the muscle of the unexposed side continues to contract, and it is inhibited by stimuli just as when both sides are intact.

Reflex inhibition of the caudal heart muscles by sensory stimulation.—Inhibitions of the rhythm of the caudal heart muscles may also be easily brought about by cutaneous stimulation. The result is the same in whatever region the skin be stimulated, whether on the tail near the caudal heart, or on the head at the farthest point away from the heart. By electrical stimulation of the skin I succeeded in producing reflex inhibitions of the caudal heart muscles with stimuli

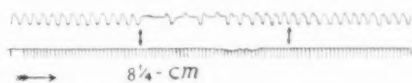


FIGURE 7. Experiment July, 1890. Original size. Partial and unequal inhibition of the caudal heart muscles produced by a moderate stimulation of the peripheral end of the spinal cord. The stimulation began at the first (left) arrow and ended at the second. Secondary coil $8\frac{1}{4}$ cm. from the primary. Time in seconds.

that were too weak to arouse contractions of the lateral muscles, *i. e.*, too weak to arouse general movements of the body. Once, also, while I was observing the contractions of the caudal heart muscles in a hagfish resting in a glass aquarium a second hagfish swam slowly by and touched the skin on the head of the quiet one. Instantly the contractions of the caudal heart muscles ceased although the animal gave no other visible response to the touch. The inhibition lasted for a few seconds only and resembled in general character the inhibitions produced by stimulating the spinal cord. This instance is worthy of note as a case of inhibition due to natural cutaneous stimulation.

A reflex inhibition of the heart muscles is brought about by stimulation of the exposed muscular surface of the subcutaneous sacs after the skin has been removed. This particular experiment was tried only in the caudal region near the muscles. A possible direct influence of the current on the muscles was suspected. But when the nervous connections of the point stimulated were cut, or the cord sectioned between the point stimulated and the heart muscles, then stimulation of the same point was without influence upon the rhythm of the heart muscles. This experiment is of importance as showing the presence of afferent fibres in the inner wall of the subcutaneous sacs, fibres which may possibly be influenced by variations in the pressure of the blood in these spaces. (See page 376.)

An automatic caudal heart centre in the spinal cord. — In seeking to demonstrate the mechanism by which the rhythmic contractions of the caudal heart muscles are inhibited under the different conditions mentioned above, several fundamental observations were recorded.

In the first place attempts to show that rhythmic contractility is a property of these particular muscles were wholly futile. Rhythmic contractions were never re-established in the muscles after isolation from the body, although tone changes occurred under the influence of inorganic salt solutions. The muscles remain alive in the moist chamber and respond to electrical stimuli for several hours. However, as stated above, the muscles contract rhythmically in the isolated tail. But the isolated tail contains the caudal end of the spinal cord with the associated spinal nerves. By cutting away successive bits of this cord I reached the following very interesting results: —

1. The muscles continue in undisturbed rhythm until all the cord

is destroyed except that portion lying immediately dorsal to the muscles themselves. 2. Partial destruction of the cord just dorsal to the muscles, results in a disturbance of the rate, and a decrease in the force of the contractions. The destruction of the posterior half of this small bit of cord seems to produce more marked disturbances than does the destruction of the anterior half. 3. Total destruction of the cord, *i. e.*, *complete isolation of the caudal heart muscles from the spinal cord, is followed by total interruption of the rhythmic contractions.* In this condition of isolation the muscles retain their irritability as shown by their responses to single induction shocks and to tetanization.

The above results point to the inevitable deduction that the rhythmic contractions of the caudal heart muscles is not due to an inherent property of the muscles themselves, as in true cardiac muscle, but is due to rhythmic discharges of motor impulses from the spinal cord itself. *There is an automatic caudal heart centre located in the spinal cord which discharges rhythmic motor impulses to the caudal heart muscles.* This centre is located in that portion of the spinal cord which is immediately dorsal to the heart itself. It is a rather diffuse centre and probably extends over at least three or four metameres or segments of the cord, as indicated by partial destruction of this part of the cord.

The cessation of the contractions of the muscles following stimulation of the sectioned cord is to be explained as a reflex inhibition of the automatic nerve centre. Likewise inhibitions accompanying body movements, or resulting from cutaneous stimulation, or caused by stimulation of the wall of the subcutaneous sacs, are all explained on the same ground as inhibitions of this centre. We have here a vascular mechanism which in its nerve-motor relations rather closely resembles the respiratory apparatus in higher forms. In the caudal heart centre in the tail of *Polistotrema*, as in the respiratory centre of the medulla of mammals, there is a rhythmic discharge of motor impulses, and this rhythmic activity may be reflexly inhibited by afferent impulses.

SUMMARY.

The new facts set forth in this paper may be briefly summarized as follows: —

1. *Polistotrema stouti* possesses a vascular organ in the tail which has the function of, and possesses the significance of a vascular heart, the caudal heart. First discovered in *Myxine*, by Retzius.

2. The caudal heart consists of a paired sac, each cavity of which receives blood from a median caudal sinus and discharges it into the caudal vein through openings that are guarded by special valves. Each side possesses a special muscle, the *musculus cordis caudalis* of Retzius.

3. The caudal heart acts on the principle of a double force-pump, the *musculi cordis caudalis* in their rhythmic alternate contractions producing alternate decreased pressure upon and consequent filling of the sac on one side, and at the same time increased pressure and emptying of the opposite sac.

4. The rhythmic contractions of the *musculi cordis caudalis* are produced by rhythmic discharges of motor impulses which proceed from an automatic caudal heart centre in the caudal end of the spinal cord.

5. The caudal heart centre in the cord may be inhibited reflexly by stimulation of the skin at any point, or by stimulation of the cord anterior to the centre. Inhibitory impulses to the centre are normally associated with the discharge of motor impulses to the lateral muscles.

6. The inhibitions of the rhythmic discharges from the caudal heart centre which result from moderate faradaic stimulation of the peripheral end of the sectioned cord are coincident with the stimulus. With excessively strong stimulation the inhibition may last longer than the stimulus. With weaker stimulation the rhythm may be again established during stimulation, or only a slight slowing of the rate of the contractions may result.

STANFORD UNIVERSITY, California,
October 15, 1899.

ON THE DIFFERENT EFFECT OF IONS UPON MYOGENIC
AND NEUROGENIC RHYTHMICAL CONTRACTIONS
AND UPON EMBRYONIC AND MUSCULAR TISSUE.

BY JACQUES LOEB.

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I. ON THE DIFFERENT EFFECT OF IONS UPON THE MARGIN AND
THE CENTRE OF A HYDROMEDUSA (GONIONEMUS).

IN a preceding paper¹ I gave a number of facts which force us to assume that not the salts themselves are in combination with the proteids but their ions, and that the physical qualities of the various ion proteids are different. This being true, a pure solution of an electrolyte ought to be poisonous, and I have been able to prove that a pure NaCl solution of the strength in which marine animals live kills them in a comparatively short time. The addition of a small amount of certain other metal ions (K and Ca) renders the solution harmless. This supports the assumption *that irritability depends upon the various ions, especially the metal ions (Na, Ca, K, and Mg) existing in definite proportions in the tissues.* But as each tissue has its own specific irritability it would follow that various tissues must possess the various ions in different proportions. This paper contains the results of a series of investigations on this subject.

Gonionemus propels itself by rhythmical contractions of its swimming bell. The swimming bell, however, does not contract continuously, like the heart, but in groups of rhythmical contractions, followed by longer pauses. The swimming bell of the Medusa may be divided into two regions, a marginal region containing the double nerve ring and its ganglia, and the central region which has no ganglia, but is said to possess scattered ganglion cells. The case is similar to that of the heart, which has ganglia in the auricles and sinus venosus, whose ventricle is however free from ganglia but contains scattered ganglion cells.

¹ LOEB, J.: This journal. 1899, iii, p. 327.

Romanes first stated that if we cut a Hydromedusa in two the marginal part with the ganglia will continue to beat rhythmically very much like the whole Medusa, while the centre ceases to beat. These results have been confirmed by several authors, but I have found that the statement of Romanes is correct only for sea water. If the centre of a Hydromedusa be put into a pure $\frac{5}{8} n$ NaCl or $\frac{5}{8} n$ NaBr solution it begins to beat rhythmically for an hour immediately after the operation. Hence the centre as well as the margin is capable of spontaneous contractions. But why does the centre not beat rhythmically in sea water? If it be put into a solution of 98 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{1}{3} n$ CaCl₂ it no longer beats rhythmically. The same is true for a solution of 98 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{5}{8} n$ KCl, or a solution of 96 NaCl + 2 CaCl₂ + 2 KCl. Hence the Ca- and K- ions of the sea water prevent the centre from beating rhythmically.

This harmonizes with my previous experiments on the muscles of the skeleton.¹ The latter are able to beat rhythmically in a pure NaCl or a NaBr solution or any solution with Na-ions. But a small addition of Ca-ions or K-ions or both prevents rhythmical contractions. We owe it to the presence of these ions in our blood that our muscles do not contract rhythmically like the heart.

Thus we see that there is a typical difference between the effects of ions on rhythmical contractions originating in the muscles directly and those originating in parts which contain ganglia or which originate in the latter themselves. *Inasmuch as the whole Gonionemus beats in the rhythm of the margin and inasmuch as the whole Gonionemus is just as immune against the Ca- and K-ions of the sea water as the margin, it follows that the normal contractions of the Gonionemus originate in the part which contains the ganglia. It is probable moreover that the margin and the centre must contain the three metal ions (Na-, Ca-, and K-) in different proportions.* That this is only a difference in degree, however, is proved by the fact that an increase in the amount of K- and Ca-ions above that of the sea water will finally stop the rhythmical contractions of the margin. On the other hand it is probable that a very small amount of K- and Ca-ions, smaller than that in the sea water, allows the centre to beat rhythmically.

This difference between the margin and the centre is not the same in all Medusæ. If we cut off the margin in an Acalepha (for instance

¹ Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln hervorrufen. Festschrift für Fick. Braunschweig, 1899.

Aurelia aurita) the centre begins to beat in sea water a short time after the operation. It is possible that a comparative study of the heart-beat would reveal similar facts.

We have thus far shown that the centre of a *Gonionemus* is able to beat for about an hour in a pure NaCl solution while the whole *Gonionemus* or the margin is able to beat in a NaCl solution containing in addition a small amount of K- and Ca-ions. How does a whole *Gonionemus* behave in a pure NaCl solution? As stated above the contractions of *Gonionemus* occur in sea water in groups followed by long pauses. If a *Gonionemus* be put into a $\frac{5}{8}$ *n* NaCl solution the swimming bell contracts without interruption and the rate of contraction increases considerably. It may within two minutes reach a rate of 200 contractions per minute, but soon ceases to beat. If the $\frac{5}{8}$ *n* NaCl solution be diluted with distilled water the increase in the rate of contractions occurs more slowly and the contractions continue longer. If we use a solution of 98 c.c. $\frac{5}{8}$ *n* NaCl + 2 c.c. $\frac{1}{8}$ *n* CaCl₂ it contracts much more slowly but the contractions last longer. In a solution of 98 c.c. $\frac{5}{8}$ *n* NaCl + 2 c.c. $\frac{5}{8}$ *n* KCl it does not beat at all with the exception of a few contractions at the beginning. In a solution of 96 c.c. $\frac{5}{8}$ *n* NaCl + 2 c.c. $\frac{5}{8}$ *n* KCl + 2 c.c. $\frac{1}{8}$ *n* CaCl₂ it beats very slowly but much longer than in any other of the solutions mentioned. In pure $\frac{5}{8}$ *n* KCl or $\frac{1}{8}$ *n* CaCl₂ solutions no contractions occur.

The explanation of all these facts seems to me to be as follows: If a *Gonionemus* be put into a pure NaCl solution, Na-ions begin to enter the tissues. As soon as they contain a certain number of Na-ions any further increase of the Na-ions raises the rate of contractions. On the other hand the substitution of Ca- and K-ions for Na-ions has the opposite effect (as long as not too many Na proteids are formed). If too many Na-ions have entered into combination with the proteids the irritability ceases. We shall see later that in this case the substitution of Ca- or K-ions for Na-ions restores the irritability.

Thus the Na-ions play an important rôle in the rhythmical contractions. It is just as necessary that a certain number of Na proteids exist in the tissues of the Gonionemus as that a certain number of Ca and K proteids be present. The proportion of these three proteids is however apparently different in the margin and in the centre. In both kinds of tissue the relative number of Na proteids is greater than that of the other proteids.

This view differs from the one generally held in connection with

the heart-beat that the NaCl in the blood serves mainly the purpose of preventing the tissues from losing or taking up water,¹ while the Ca salts are considered as the cause of the systole and the KCl is said to favor the diastole of the heart. The fact that a Medusa not only contracts rhythmically in a pure NaCl solution but beats much more rapidly in such a solution than in sea water, shows that neither the Ca- nor K-ions of the surrounding medium are directly necessary for the systole or diastole. If they have any effect they only diminish the rate of contraction (besides maintaining the contractility much longer). But the above mentioned erroneous conception concerning the rôle of the three ions can be proved in another way. I had solutions of cane sugar and glycerine prepared which were isosmotic with a $\frac{5}{8}$ *n* NaCl solution. The following solutions were tried:

- | | | | | | |
|----|-------------------------------|---|-----------------------------------|---|---|
| 1. | 96 c.c. distilled water | + | 2 c.c. $\frac{5}{8}$ <i>n</i> KCl | + | 2 c.c. $\frac{1}{8}$ <i>n</i> CaCl ₂ |
| 2. | " cane sugar | + | " " | + | " " |
| 3. | " glycerine | + | " " | + | " " |
| 4. | " $\frac{5}{8}$ <i>n</i> LiCl | + | " " | + | " " |
| 5. | " $\frac{5}{8}$ <i>n</i> NaCl | + | " " | + | " " |
| 6. | " $\frac{5}{8}$ <i>n</i> NaBr | + | " " | + | " " |

In the first four solutions no rhythmical contractions occurred after the first minute. In the fifth and sixth solutions the rhythmical contractions continued for several hours. If it were true that the NaCl serves only to maintain the osmotic pressure while the Ca produces the contractions, we ought to expect that the *Gonionemus* would contract just as well in the glycerine or sugar or LiCl solution as in the NaCl solution. I have made in addition to these a number of other experiments, all of which prove that only in solutions of electrolytes (especially Na salts) is the *Gonionemus* able to contract rhythmically. The belief that calcium is the stimulus that produces the heart-beat is based upon another observation which I think was first made by Howell and his pupils.² When a heart stops beating in Ringer's solution it begins to beat again (for a little while) in a solution which contains more Ca. It is easy to confirm this observation for *Gonionemus*. If a *Gonionemus* is thrown into a solution of 98 c.c. $\frac{5}{8}$ *n* NaCl + 2 c.c. $\frac{1}{8}$ *n* CaCl₂ or a pure $\frac{5}{8}$ *n* NaCl solution, it stops contracting after a certain time but beats again for a little if thrown into a solution with more CaCl₂, for instance 95 c.c. $\frac{5}{8}$ *n* NaCl + 5 c.c. $\frac{1}{8}$ *n* CaCl₂. This seems to favor the assumption that the Ca-ions are the stimulus for the contraction of the swimming bell of a Medusa. But a simple control

¹ HOWELL: This journal, 1898, ii, p. 47.

² HOWELL: *loc. cit.*

experiment shows that this assumption is erroneous. If we throw a *Gonionemus* first into the stronger solution, for instance, of 95 c.c. $\frac{1}{2}$ *n* NaCl + 5 c.c. $\frac{1}{2}$ *n* CaCl₂, and wait until it stops contracting, it will begin to contract again if we put it back either into the solution with less CaCl₂ (for instance, 98 c.c. $\frac{1}{2}$ *n* NaCl + 2 c.c. $\frac{1}{2}$ *n* CaCl₂) or into a pure NaCl solution. The true explanation of this phenomenon is, I believe, as follows: In the pure NaCl solution or the solution with little CaCl₂, too many Na-ions combine with the proteids and this leads to a loss of irritability. If the *Gonionemus* be brought into a solution with more Ca- and less Na-ions some Ca-ions will take the place of Na-ions in the tissues and this restores the irritability. But finally too many Ca-ions enter and the physical qualities are changed again, thus making the *Gonionemus* inirritable. If the same *Gonionemus* then be put into a pure NaCl solution or into a NaCl solution with fewer Ca-ions the Na-ions will take the place of some of the Ca-ions and this will restore the irritability.

We thus arrive at the conclusion that the rhythmical contractions of *Gonionemus* depend upon the presence of Na-, Ca-, and K-ions in definite proportions in the ion proteids of the tissues. These proportions evidently differ in various kinds of tissues. Myogenic contractions are prevented by a smaller amount of K- and Ca-ions in the surrounding NaCl solution than neurogenic contractions or contractions originating in parts containing ganglia.

ON THE DIFFERENT EFFECTS OF IONS UPON UNDIFFERENTIATED EMBRYONIC TISSUE AND UPON MUSCLE.

While the method established in the preceding chapter may be successfully applied to all kinds of tissues, I was most interested to know whether there is a marked difference between undifferentiated embryonic and differentiated older tissue. By embryonic protoplasm or tissue I mean the early egg cells, the growing regions in plants and animals, rapidly growing tumors, regenerating parts or organs; in short, cells which are characterized by rapid multiplication. If we are ever to build up a technical or constructive in the place of a merely analytical biology we shall be able to do it on the basis of a more thorough knowledge of the character of embryonic matter. I tried to find out whether the various metal ions have the same effect upon the undifferentiated egg cells as upon muscle. These experiments throw some light upon another problem. The karyokinetic cell divi-

sion has been identified with phenomena of muscular contraction. We shall see incidentally how far such an idea is justifiable.

In my former experiments on development I was guided by the idea that the various morphological stages were preceded by chemical changes. In order to see how much justification there is for this idea I tried to discover whether lack of oxygen or an increase in the concentration of sea water affects the embryo differently in different stages of its development.¹ I used for these experiments the eggs of a marine fish (*Fundulus*). The eggs of this fish complete their development in about two weeks (at the proper temperature). We may discriminate three stages in the development of this fish. The first consists solely of processes of cell division and the expansion of the blastoderm. This stage lasts about twenty-four hours. It is followed by the formation and beginning differentiation of the embryo during the second twenty-four hours. The third stage of development begins with the establishment of muscular activity, especially the heart-beat, about seventy-two hours after fertilization.

If newly fertilized eggs be exposed to a partial oxygen vacuum the development may go on for some time (about twenty-four hours). The eggs, however, may remain alive in the oxygen vacuum for four days. If after that time they are put back into normal sea water they develop into normal fish which hatch in due time. If we put an embryo which is three days old into the same oxygen vacuum it loses its power of development within twenty-four hours. The older the embryo the more deleterious is the lack of oxygen. This is only comprehensible on the assumption that the morphological differentiation is accompanied or preceded by changes in the chemical constitution of the embryo.

The same result was obtained in experiments in which the concentration of sea water was raised by the addition of NaCl, but curiously enough in this case the younger embryo was more sensitive to an addition of NaCl to sea water than the older embryo. An addition of five grams of NaCl to 100 c.c. of sea water did not prevent the development of the *Fundulus* egg, but an addition of ten grams of NaCl to 100 c.c. of sea water prevented the formation of an embryo. Newly fertilized eggs began to segment in such a solution but stopped very soon and lost their power of development permanently within from six to ten hours. A germ that was allowed to develop during the

¹ LOEB: *Archiv f. d. ges. Physiol.*, 1894, iv, p. 530.

first twenty-four hours *in normal sea water* withstood a solution of sea water to which 10 per cent of NaCl had been added much better. In such a solution it could go on with its development for several days; in some cases as long as ten to fourteen days. An embryo which had been allowed to develop in normal sea water until its circulation was established (third or fourth day) was even able to live for several days in sea water to which 24 per cent NaCl had been added. When I made these experiments I still accepted the common view that NaCl was an indifferent substance and that in these experiments it acted only osmotically. The results of my recent work and of experiments to be mentioned in this paper however prove that we have to deal with the effects of Na- and Cl-ions in these experiments. It therefore follows that the Na- and Cl-ions (especially the former) are more injurious during the earliest stages of cell division than during the later stages. I made my new experiments on the effects of various ions upon development on the eggs of the same form.

If the eggs of *Fundulus* be put into a $\frac{1}{2} n$ NaCl solution immediately after fertilization the development stops in most cases at an early stage (64 to 128 cells) and only a few eggs form an embryo. If the development does not stop during the first twenty-four hours it continues as a rule normally for one or more weeks. Hence a pure NaCl solution seems to be more poisonous during the first twenty-four hours of development than during the later stages. Control experiments verify this assumption. Eggs that are allowed to develop the first eighteen or twenty-four hours in sea water and are then put into a $\frac{1}{2} n$ NaCl solution continue to develop in almost every case. No embryo is able to hatch in these solutions.

In our previous paper we showed that a young fish died in a few hours in such a solution. The reason that the egg lives longer in it may be due either to the fact that the tough egg membrane does not allow the ions to penetrate so fast into the embryo, or to the presence of the yolk which to a certain extent may regulate the proportion of ions in the embryo.

If we dilute a $\frac{1}{2} n$ NaCl solution with distilled water we find that in a $\frac{1}{4} n$ NaCl solution all the newly fertilized eggs may form an embryo. Some of the embryos even hatch in such a solution. Their duration of life after hatching is, however, very short. The fact that the eggs of *Fundulus* are able to develop in sea water to which as much as 5 per cent NaCl has been added shows that other constituents of the sea water are able to counteract the poisonous effects of a pure NaCl

solution. In a solution of 98 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{5}{8} n$ KCl only a small number of embryos are formed. They cannot be kept alive for more than a week. In a solution of 98 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{5}{8} n$ CaCl₂ every egg develops, but only in exceptional cases does the fish hatch. Those that hatch die immediately afterwards. The addition of even as little as $\frac{1}{2}$ c.c. $\frac{1}{8} n$ CaCl₂ to 100 c.c. $\frac{5}{8} n$ NaCl causes all the eggs to develop. A small amount of Ca-ions counteracts the poisonous effect of a large quantity of Na-ions sufficiently to allow the development to go on, but not enough to allow the embryos to hatch. In a solution of 96 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{1}{8} n$ CaCl₂ + 2 c.c. $\frac{5}{8} n$ KCl all the eggs not only develop but the young fish hatch and live indefinitely.

In distilled water all the eggs are able to develop and the young fish hatch in due time and live indefinitely. Hence the Ca- and K-ions of the above mentioned solution are not directly necessary for the development of the fish. They are only indirectly necessary to counteract the poisonous effects of the Na-ions in a solution of a Na salt. In a glycerine solution of the same osmotic pressure as a $\frac{5}{8} n$ NaCl solution, no embryo was formed. In mixtures of glycerine and sea water embryos formed but the glycerine acted as a poison; the more glycerine the solution contained, the quicker it killed them.

Thus far our results agree entirely with our previous results on the poisonous character of a pure NaCl solution. Such a solution has a poisonous effect on the germ of *Fundulus*, and the Na-ions are exclusively or mainly responsible for the poisonous effect. But this poisonous effect is much more marked during the first twenty-four hours of development than during the later stages.

Everyone who has had experience with the effects of KCl upon the contraction of muscles knows how poisonous a pure KCl solution is. I was much surprised to find that a $\frac{5}{8} n$ KCl solution is even less harmful to the newly fertilized egg of *Fundulus* than a $\frac{5}{8} n$ NaCl solution. More eggs develop in the former than in the latter solution. The following table gives a clear illustration of this condition: Mixtures of $\frac{5}{8} n$ NaCl and $\frac{5}{8} n$ KCl solutions were used. Each solution had about 70 to 80 eggs. The percentage of eggs that formed embryos is indicated for each solution.

TABLE I.

	Character of Solution.				Percentage of eggs that formed embryos.
1	98 c.c. $\frac{1}{2} N$ NaCl +	2 c.c. $\frac{1}{2} N$ KCl			6 per cent.
2	95 "	+ 5 "			10 "
3	90 "	+ 10 "			25 "
4	60 "	+ 40 "			30 "
5	40 "	+ 60 "			40 "
6	10 "	+ 90 "			33 "
7	5 "	+ 95 "			33 "
8	2 "	+ 98 "			33 "
9	0 "	+ 100 "			38 "

It is evident that if the number of K-ions is greater than the number of Na-ions the percentage of eggs which are able to develop is larger. It is remarkable that in a solution of 98 NaCl + 2 KCl fewer eggs form an embryo than in 95 NaCl + 5 KCl or in 90 NaCl + 10 KCl, although in these last two solutions the amount of KCl is far in excess of that in sea water. Hence we are forced to conclude that K-ions are less poisonous for the earlier stages of *Fundulus* than Na-ions. It is better for the egg that K-ions enter into combination with the proteids of the protoplasm than Na-ions.

As soon, however, as the heart begins to beat and circulation becomes necessary for the embryo, the K-ions become more poisonous than the Na-ions. Only in the first three solutions is the heart of the embryo able to beat for a few days. But even in these solutions no embryo lives longer than about a week. In the other solutions the embryos die much earlier. This should certainly serve us as a caution in taking it for granted that the cell division is due to contractile phenomena of the same order as those occurring in the muscle.

Ca-ions are in small quantities more beneficial, in larger quantities more injurious, than Na-ions. The addition of a little $\frac{1}{8} N$ $CaCl_2$ to a pure NaCl or a pure KCl solution causes all the eggs to develop, but very soon a limit in the addition of $CaCl_2$ is reached where no more embryos are able to form. The following table shows this in a very marked way: —

TABLE II.

	Character of Solution.	Percentage of eggs that formed embryos.
1	100 c.c. $\frac{1}{8} n$ KCl + 0 c.c. $\frac{1}{8} n$ CaCl ₂	15 per cent.
2	98 " + 2 "	100 "
3	95 " + 5 "	100 "
4	90 " + 10 "	100 "
5	75 " + 25 "	100 "
6	60 " + 40 "	9 "
7	50 " + 50 "	0 "

In none of these solutions was an embryo able to hatch or to live through the whole period necessary for development. The K-ions in these solutions caused a cessation of the heart-beat. The more K-ions the solution contained the sooner this happened. In mixtures of Na- and Ca-ions the limit where Ca-ions prevent the formation of an embryo is still lower. As a rule in a mixture of 75 c.c. $\frac{1}{8} n$ NaCl + 25 c.c. $\frac{1}{8} n$ CaCl₂ no embryo is formed.

It has long been maintained that there is an antagonism between Ca- and K-ions. The following experiment demonstrates this relation in a very striking way. If we mix 75 c.c. of sea water with 25 c.c. $\frac{1}{8} n$ CaCl₂ as a rule only a very small percentage of the eggs are able to form an embryo. But if we add a large quantity of KCl almost every egg forms an embryo. In two experiments not a single embryo was formed in a mixture of 75 c.c. of sea water + 25 c.c. $\frac{1}{8} n$ CaCl₂ while in 50 c.c. of sea water + 25 c.c. $\frac{1}{8} n$ CaCl₂ + 25 c.c. $\frac{1}{8} n$ KCl, every egg formed an embryo. I have repeated this experiment very often and in each case obtained similar results. *But while the K-ions antagonized the poisonous effects of the Ca-ions upon the formation of the embryo, the poisonous effects of the K-ions upon the heart-beat were not counteracted by the Ca-ions.* In none of the embryos formed in this solution was the heart able to beat sufficiently long to enable the embryo to complete its development. As a rule on the sixth day every egg was dead.

In a pure $\frac{1}{8} n$ CaCl₂ solution the germ died in the early stages of segmentation (4 to 8 cell stages). It appeared to be coagulated.

Not even in a mixture of equal parts of such a solution with distilled water was a single embryo formed. In a solution of 25 c.c. $\frac{1}{8}n$ CaCl_2 with 75 c.c. of distilled water embryos could be formed. In one case as many as 30 per cent of the eggs contained embryos. This is the more remarkable as in a mixture of 75 c.c. of sea water + 25 c.c. $\frac{1}{8}n$ CaCl_2 a much smaller percentage of eggs formed embryos. In a $\frac{1}{8}n$ CaCl_2 solution all the eggs formed embryos whose development was normal.

We should expect that if we put eggs immediately after fertilization into a pure solution of a Na salt whose anion precipitates calcium the fatal effects of the Na-ions upon the development would be still more obvious than in a pure NaCl solution. The precipitation of Ca-ions in the protoplasm would accelerate the disproportion between the Na- and Ca-ions of the protoplasm. I tried $\frac{1}{8}n$ Na_2SO_4 solutions. In no experiment was a single embryo formed and in each case the development of the germ stopped in an earlier stage than in the pure NaCl solution. This corroborates our view that the poisonous character of the pure NaCl solution is due to the fact that for the development of the egg the Na-, Ca-, and K-ions must exist in definite proportions in the protoplasm.

In a pure $\frac{1}{8}n$ MgCl_2 solution no egg can develop. Even in equal parts of $\frac{1}{8}n$ MgCl_2 and distilled water only a small proportion of eggs (20 per cent) were able to form embryos, none of which hatched. Mg-ions behave toward the egg of *Fundulus* very much like Ca- and unlike K-ions. In a solution of 98 c.c. $\frac{5}{8}n$ NaCl + 2 c.c. $\frac{1}{8}n$ MgCl_2 all the eggs form embryos, although no fish hatch. But in larger quantities the Mg-ions are not so poisonous as the Ca-ions. Even in a mixture of equal parts of $\frac{5}{8}n$ NaCl + $\frac{1}{8}n$ MgCl_2 as many as 75 per cent of the eggs form embryos (although none of the latter hatch). This behavior of the Mg-ions is similar to the one described in my paper on the absorption of liquids. The above mentioned experiments on the effects of K-ions show very clearly that the effect of these ions upon cell division is altogether different from their effect upon the rhythmical contractions. This is not only true for the cells of *Fundulus* but also for the egg cells of the sea urchin. I intend to discuss the effect of ions upon the cell division in the eggs of sea urchins in the next paper.

SOME GENERAL CONCLUSIONS.

1. The results of this paper bear upon several other problems which we have thus far had no chance to discuss sufficiently. There has been a controversy as to whether the contractions of the heart are myogenic or neurogenic. The problem is the same as for *Gonionemus*. In the latter it is certain that under ordinary circumstances (*i. e.* in the presence of the K- and Ca-ions of the sea water), the impulses for the rhythmical contractions originate in the nervous system or at least in the margin. It is however not the histological or morphological structure of the ganglia which allows them to be so important, but their chemical constitution. The centre of *Gonionemus* is able to beat rhythmically in a pure NaCl or NaBr solution. It is true that the centre of *Gonionemus* (and the apex of the heart) contain single scattered ganglion cells. One might think that these latter are responsible for the rhythmical contractions of the centre which occur in pure NaCl or NaBr solutions. But the muscles of the skeleton (even if curarized) show rhythmical contractions in the same pure NaCl or NaBr solutions provided the latter do not contain any K- or Ca-ions.

2. It would be unwarranted to say that Ca- or any other ions are the cause of, or the stimulus for, the rhythmical contractions in *Gonionemus*, or the heart or any other organ. It would be much nearer the truth to assume that for the possibility of rhythmical contractions the Na-, Ca-, and K-ions must exist in definite proportions in the tissue which is expected to show rhythmical activity. Only so long as these proportions are preserved does the tissue possess such physical properties and such labile equilibrium as to be capable of rhythmical processes or contractions. If the tissue has permanently or temporarily more Ca- and fewer Na-ions than are required for the above mentioned physical properties and condition of equilibrium, an increase of Na-ions in the tissue will cause rhythmical contraction. In such a case the tissue will begin to contract rhythmically or beat at an increased rate in a pure NaCl solution. If the tissue, however, contains too many Na- and too few Ca-ions a further increase of the latter in the tissue will cause the beginning of rhythmical contractions. In this case the addition of Ca-ions to a pure NaCl solution will produce rhythmical activity. In a former paper I have shown that skeletal muscle can be caused to beat rhythmically if we increase

the number of its Na-ions without increasing the number of its Ca-ions. In one of the next papers it will be proved that the same result can be obtained more rapidly if we decrease the number of Ca-ions in the muscle by precipitating them.

3. The phenomena of muscular contractility and the phenomena of cell division are considered by many authors as being of the same order. The rays of the astrosphere are said to be contractile fibrils which pull the chromosomes apart and accomplish the division of the mother cell into two daughter cells. I do not see how we can harmonize this hypothesis with the fact that enormous quantities of K-ions in no way interfere with the process of karyokinesis, while even a much smaller amount of K-ions annihilates muscular activity in a very short time. In the preceding paper I mentioned the fact that the ciliary motion of the blastulae of the sea urchins continues in the presence of enormous quantities of K-ions. The riddle of contractility is still unsolved. It yet remains to be proved that the ciliary motion and cell division are due to contractile processes identical with those in the muscle. Our experiments on the effects of K-ions should warn us against taking such an identity for granted.

4. While a solution of NaCl with a small amount of K- and Ca-ions allows all the various vital processes to go on (except such special phenomena as the formation of the skeleton, with which we shall deal in the next paper), we find other combinations of ions which enhance some of the vital processes while they prevent others. The most important combination in this direction is the mixture of $\frac{5}{8}$ *n* KCl with a small amount of $\frac{1}{8}$ *n* CaCl₂. In such a solution the first stages of the development of the *Fundulus* egg occur in a normal way. The fact that such a solution does not contain any Na-ions raises the question whether the main importance of ions in these phenomena does not lie in the influence they have upon the physical qualities of the protoplasm (absorption of liquids, state of matter, etc.). If this were the case we might easily understand that various mixtures of ions might bring about the same effect upon tissues provided that they affect the physical qualities of the protoplasm in the same manner. In the next paper we shall show that the eggs of the sea urchin can reach the blastula stage in a mixture of $\frac{1}{8}$ *n* MgCl₂ and $\frac{1}{8}$ *n* CaCl₂. But each of these vicarious mixtures serves only for a certain class of vital processes while a mixture of NaCl with a small amount of Ca- and K-ions allows the whole cycle of life phenomena (with certain exceptions) to be completed.

5. Herbst¹ has tried to prove that practically every substance contained in the sea water is necessary for the development of the egg of the sea urchin. His proof consisted chiefly in removing one of the constituents of the sea water, and showing that in such modified sea water the eggs were not able to develop. This method does not warrant the conclusions Herbst has drawn from them. In a solution of 96 c.c. $\frac{5}{8}$ *N* NaCl + 2 c.c. $\frac{1}{8}$ CaCl₂ + 2 c.c. $\frac{5}{8}$ *N* KCl all the *Fundulus* eggs develop and hatch. If we remove the Ca-ions the majority of *Fundulus* eggs cannot develop and of the few that develop none hatch. According to Herbst it would follow that the surrounding medium must contain Ca-ions for the development and hatching of the *Fundulus* eggs. Yet we have seen that the *Fundulus* egg develops and hatches in distilled water. Ca-ions become a necessity only if the surrounding solution contains Na-ions in excessive quantities.

¹ HERBST: Archiv für Entwicklungsmechanik, 1897, v, p. 649.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR
ORGANISMS. — VI. ON THE REACTIONS OF
CHILOMONAS TO ORGANIC ACIDS.

By H. S. JENNINGS.

IN the January number of this Journal appeared two papers dealing with the reactions of infusoria.¹ Both the papers treat, among other matters, somewhat fully of the reactions of *Chilomonas*, but from a different standpoint in each case. Garrey's paper considers the subject chiefly from a chemical standpoint, giving an account of the chemical substances in solutions of which *Chilomonas* forms aggregations, and of other substances which the animals leave vacant, and determining the exact factors in the solutions to which these results are due. Garrey did not, however, work out the mechanism of the reactions, — that is, he did not determine the exact movements of the animals when stimulated. My own paper, on the other hand, dealt with the matter from the side of the organism, and was concerned especially with the mechanism of the reactions, showing the exact movements of the animals under stimulation. I did not, however, treat of the special chemical substances in which aggregations are formed, or which are left vacant, by the organisms. The direct application of the reaction mechanism which I have described to the phenomena of aggregation, etc., described by Garrey has, therefore, not been made. As the conception presented by Garrey of the nature of the activities of the organism in these reactions is different from that to which I was led by a study of the reaction mechanism, it seems important to make this application. I am convinced that there is no contradiction between the observations of Garrey on this point and my own observations, but that on the contrary the two supplement each other neatly and lead to the same conclusions.

The organism concerned is the small Flagellate *Chilomonas*. Brief descriptions of the organism are given in both the papers above-

¹ (1) The effect of ions upon the aggregation of flagellated infusoria, by Walter E. Garrey: This journal, iii, no. vi, pp. 291-315. (2) Studies, etc. V. On the movements and motor reflexes of the Flagellata and Ciliata, by H. S. Jennings: This journal, iii, no. vi, pp. 239-260.

mentioned; one point which becomes of much importance for an understanding of the essential nature of the reactions was not specifically mentioned in either paper, however. This is the well-known fact that *Chilomonas* is an unsymmetrical animal, the so-called "upper," or larger lip lying at the dorso-dextral angle of the body, as figured by Bütschli, in Fig. 9b of Plate XLV, in his great work on the Protozoa, — so that the organism cannot be divided either dorso-ventrally or dextro-sinistrally into similar halves, and it is impossible to speak of "symmetrical points on the surface of the body," as is done in bilaterally symmetrical organisms.

Garrey finds that certain chemicals "cause the organism to become restless, very swift shooting movements being caused." As a result of these movements the organisms soon leave the area of operation of the chemical causing the reactions. This phenomenon Garrey calls "chemokinesis." Expressed in the more precise terms of the "motor reflex" described in my own paper (*loc. cit.*), this is as follows. These chemicals cause in a marked degree the motor reflex of *Chilomonas*; this reflex consists of the following activities. The animal darts *backward*, turns sideways *toward the smaller lip*, then swims forward in the new path thus determined by the position of the smaller lip. This being repeated, the animal is in time brought out of the region of the agency causing the motor reaction. The whole phenomenon is evidently precisely analogous to the conduct of *Paramecium* in leaving vacant a drop of some chemical to which it is "negatively chemotactic" (or chemokinetic). The only difference between the two cases seems to be that *Chilomonas* forms a ring around the outside of such a drop, while *Paramecium* does not. This is due to the fact that *Chilomonas* is normally much less active than *Paramecium*, and usually comes to rest as soon as a motor stimulus is lacking; it therefore comes to rest as soon as it gains the outside of the drop of the chemical which acts as a stimulus. *Paramecium*, on the other hand, is a strong swimmer, and once in motion, it continues to move until stopped by another stimulus; it therefore forms no collection about such a drop. — The above is given as merely supplementary to Garrey's account, and not as in any sense a correction of it: I apprehend that there would be no disagreement on these points.

In certain organic acids (acetic, butyric, lactic) and their salts Garrey finds that *Chilomonas* forms dense aggregations, — as *Paramecium* does in weak solutions of acids of any kind. The special

problem here is, how do the organisms gather in the region of the organic acid? Garrey states that "a study of the mechanics by which the organism is oriented, or by which it is prevented from moving from the ring into the stronger acid of the clear area or the weaker acid surrounding the ring, proved fruitless" in his case, so that the question as to the mechanics of the phenomenon remains an open one. In what follows, I shall set forth observations which I believe give a clear answer to this question.

In studying the reactions to organic acids (acetic and butyric were chiefly used), I have employed two methods. In one of them an apparatus similar to that described by Garrey¹ was used. This method, in which the reactions are examined in a chamber 1 to 1½ mm. deep, is well suited for showing the grosser phenomena of aggregation, since it is thus possible to work with a very large number of organisms, and the aggregations formed are dense. On the other hand, it is, of course, impossible to study the exact behavior of creatures but 40 μ long in a layer a millimetre deep. The second method was that used by me in studying the reactions of *Paramecium*: it is described and figured in the second of my *Studies*,² p. 314. In this method the animals are mounted in a very thin layer of water under a supported cover-glass, and a drop of the substance to be tested is introduced with a capillary pipette into the preparation. The aggregations of the animals are then produced in the same manner as by the first method, but of course contain comparatively few individuals, so that the gross appearance is not striking. But the thickness of the layer of water can be diminished to any desired extent by decreasing the size of the supporting glass rods, so that the individuals can be watched even with a high power lens; thus the exact behavior of the animals may be noted.

Using this second method, when a drop of weak acetic or butyric acid is introduced, the swimming *Chilomonads* behave as follows. Those which swim against the edge of the drop enter it without reaction. If they continue to swim across it, when they come to the opposite boundary, where they would, if unchecked, pass again to the outer medium, the change in the solution at this point produces the characteristic reflex, by which the animals are turned toward the smaller lip, and thus must pass back again into the drop. This may continue, so that the animal remains in the drop. Others enter in

¹ GARREY: *loc. cit.*, p. 294, Fig. 3.

² JENNINGS: *This journal*, 1899, ii, pp. 311-341.

the same way and are retained in the same way, so that the drop in time contains large numbers of individuals. Frequently an individual on entering the drop simply settles down against the slide or cover-glass, so that no motor reaction or orientation of any sort is necessary to keep it in the drop. The reason why a number of Chilomonads collect in the drop of acetic acid is then as follows. When the swimming individual comes from the outside against the drop of acid, the change in passing from the outer medium into the drop is not of such a nature as to cause a motor reflex, while *the opposite change, from the acid to the outer medium, is of such a nature as to produce the motor reflex*.¹ The further question, of why one sort of change should cause a motor reflex, while the opposite does not, is of course here, as in other motor reflexes, unanswerable; to determine what agencies will cause a motor reflex is always a matter for experiment.

As to the matter of orientation, it is, of course, evident that when a Chilomonas enters the drop, it will, as a rule, be so oriented that its anterior end is directed approximately toward the centre of diffusion of the chemical, otherwise it would not enter the drop at all. If the drop in diffusing reaches a number of resting Chilomonads, these start to swim; those which swim toward the centre of the drop are not stopped, while those which swim in other directions, coming quickly to the edge of the drop, give the motor reflex, and are therefore turned back into the drop. At a certain time then, under these circumstances, if the drop is large a considerable number of Chilomonads may be seen swimming with common orientation toward the middle region of the drop, since motion in any other direction is quickly stopped by the production of the motor reflex. Under ordinary circumstances, however, neither the individuals in the drop nor those around it will all show at a given instant a common orientation. Each organism is in a certain sense oriented whenever the motor reflex occurs, in so far as it cannot swim farther in the original direction, but may swim in some other direction. But the motor reflex does not usually occur in a large number of individuals at the same time, and it is only when this occurs that a common orientation of a large number of individuals can be expected. In spite of this usual lack of common orientation, the Chilomonads do in time form marked aggregations in the drops of acid, in the manner I have described.

¹ This is perhaps the most important point for understanding how the collections are formed: if it is not clearly apprehended the remainder of the account cannot be understood.

In the preparations 1 to $1\frac{1}{2}$ mm. thick, containing, of course, an immensely greater number of individuals coming under the influence of the stimulus at once, the Chilomonads about the diffusing solution do show a common orientation, according to Garrey: "It is easy to see that the organisms within this area are oriented with anterior ends (those bearing flagella) directed toward the ring, and that they are swimming in strictly radial lines, the lines of diffusion, toward the diffusing drop."¹ This is Garrey's ground for assuming that the aggregations are not brought about through a motor reaction such as I have described; he states that, on the contrary, these aggregations are due to "true chemotropism." As to the nature of chemotropism, he quotes with approval Professor Loeb's generalization, "The essence of chemotropic orientation would then consist in the animals placing themselves in such a position that symmetrical points on the surface of the body are cut by the diffusion lines at the same angle."

Now, in interpreting Garrey's statement as to the orientation of Chilomonas, it is necessary to recall two points not mentioned by Garrey. The first is that Chilomonas is unsymmetrical, so that no "symmetrical points on the surface of the body" are distinguishable, and second, that the animal swims in spirals of some width, and not in *straight* radial lines. The statement as to their orientation implies then only that their anterior ends are directed in general toward the centre of diffusion, — the lack of symmetry and the spiral path making any more precise statement impossible.

In the cells 1 to $1\frac{1}{2}$ millimetres deep that Garrey used the number of organisms in a given area is enormously greater than in the preparations thus far discussed, and they are not confined to a thin layer, so that it is comparatively difficult to observe individuals. Now when the diffusing acid reaches a large number of organisms, they at once start to swim in various directions. Those which swim away from the centre of diffusion quickly come to the outer boundary² of the acid and are turned through the motor reaction back into the acid area; only those which swim approximately toward the centre of diffusion (therefore in radial lines) continue on their course unhindered. Hence shortly after the beginning of the reaction a large number of animals

¹ *Loc. cit.*, p. 297.

² By "outer boundary" I mean, of course, in every case that point where the decrease in the concentration of the chemical is sufficient to be perceptible to the organism, — *i. e.*, sufficient to cause a reaction. This "boundary" is, of course, not a sharp line; it varies for different organisms.

are seen swimming toward the centre of diffusion, none in the opposite direction, while a certain number are swimming in scattered directions. Now, of course, when out of a confused movement of a large number of particles there suddenly arises a movement of a considerable proportion of the particles *in a definite direction*, the attention is immediately attracted to the definite movement, and it is only by a strong effort that the eye can be brought to attend to the individuals that are swimming irregularly in all directions. It would be necessary for me to state that my observations did not agree with those of Garrey, if by his statement that "the organisms swim toward the centre of diffusion along radial lines" he meant that all or approximately all the organisms swim in this manner. Certainly no one would make such a statement for the aggregations which occur when a drop of the acid is introduced into a preparation of the animals in a thin layer of water, where the movements of the individuals can be observed. Garrey does not state that *all* the organisms swim in radial lines, nor does he give any idea of the proportion that does so. Indeed, he gives distinctly the impression that this precise common orientation is not, as a rule, very marked (which would agree with my observations) when he says that "this migration [along radially disposed lines toward the centre of the diffusing drop] was most markedly evident in *two or three*¹ experiments in which the organisms were gathered very densely about débris situated some distance from the mouth of the diffusion tube. As soon as they came under the influence of the diffusing acid the organisms left the débris and fairly swarmed into the acid."² This observation, of course, accords perfectly with the account I have given of the way orientation takes place. Coming under the influence of the stimulus, the large number of organisms about the débris at once begin to swim (owing to Garrey's "chemokinesis," or my own "reaction to a weak stimulus").³ But only those which swim toward the centre of diffusion can continue their course; others come quickly against the boundary of the fluid and are stopped and turned (through the motor reflex) till they too finally come into a direction approximately toward the centre. Thus in a moment after the beginning of the reaction a stream of organisms is seen passing toward the centre of diffusion, as Garrey has described. (See the analogous phenomena in the case of *Paramecium* in *thermotaxis*.⁴)

¹ Italics mine.

² *Loc. cit.*, p. 310.

³ Studies, etc., V. (*loc. cit.*), p. 232

⁴ Studies, etc., II. (*loc. cit.*), p. 335.

It seems to me, therefore, that there is no disagreement between Garrey's observations and my own, in this matter. *Chilomonas* collects in regions of weak acetic or butyric acid through the agency of the motor reflex described in the fifth of my Studies, in a manner exactly analogous to the collections of *Paramecia* in drops of acid, described in the second of my Studies.¹

Whether these phenomena should be called chemotropism, or chemotaxis, or chemokinesis (the term proposed by Garrey), of course depends on the definition to be given to these words. I have hitherto used the name (chemotaxis) which was employed before the exact character of the phenomenon was known, preferring to let nature supply the definition. I find it difficult to determine whether the method of reaction described above falls within the definition of chemokinesis given by Garrey or not.

¹ *Loc. cit.*, p. 315.

NOTES ON THE INDIVIDUAL PSYCHOPHYSIOLOGY OF THE CRAYFISH.

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[From the Laboratory of Physiology in the Harvard Medical School.]

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INTRODUCTION.

EXPERIMENTS as to the different ways in which various individuals of *Cambarus affinis* Say do the same thing and as to the different methods employed by the same individual at different times, constitute the empirical basis of these notes. Of ulterior theoretic interest, there are included in the essay considerations as to the sense in which, if at all, the term *individuality* may be applied to the members of a genus as low in development as that herein examined. An article in a physiological journal is no place to discuss the philosophy of individuality, however biological the concept may be judged by some to be. It is enough here to denote one conception of the meaning of this term. We may say (avoiding all attempt at demarcation among the very lowest animal forms) that *a living organism is an individual in proportion to the relative constancy and strength of its own proper tendencies considered as manifestations of an inherent will.*

With this notion as a criterion, our theoretic inquiry will be devoted mainly toward a determination, closer perhaps than has been made, as to the degree in which correlations and constancies (which may

be said to represent "individuality" here) are present in the crayfish. Very much work has been done, of course (scientifically, only of late) on the general habits of different sorts of animals, and a certain part of this great mass of observations has been of value because at once systematic and exact. Less, much less, time has been devoted to a determination of the psychophysical habits of animals with the help of instruments of precision and exact description of small differences in their individual methods of doing things. Indeed, this sort of research is only just beginning to be conducted even on human subjects in our college laboratories and public schools. Such psychophysical studies have not only large future practical importance in their application to general education but they have also theoretic interest, as helping much toward an understanding of the relations of mind to body. To apply something of this type of method to animals whose individuality is simple enough to be somewhat definable, searching for constancy and correlation, is our present research. Temperament and mood and heredity and diathesis and other like important terms are still notions of extreme indefiniteness, which indefiniteness animal psychophysiology may perhaps help to decrease.

INCIDENTAL OBSERVATIONS ON CRAYFISH HABITS.

Method. — Specimens of *Cambarus* were chosen as subjects for this tentative experimental inquiry, for several reasons: — because their form and functions are simple but yet are developed sufficiently to exhibit a definable degree of individual variation; because their hard crustacean covering in the winter season makes the application of instruments and apparatus relatively easy and at the same time protects them from injury by the necessary constant handling; because they are easily obtained alive and readily kept in fresh water, and, being voracious eaters, are easily fed, thus maintaining their full physiologic vigor continuously; and lastly, because they are at home both in the water and out of it, thus allowing of more sorts of observation than else would be the case.

The experiments were made in the Physiological Laboratory of the Harvard Medical School chiefly during the last two months of 1899. Twenty-two adult specimens of crayfish were obtained, fifteen being females and seven males. They ranged in weight from 11.5 grams to 45 grams with an average of about 30 grams, while their lengths were from about 10 cm. to 17 cm. measuring from the end of

the abdomen to the tips of the extended chelæ. These individuals were chosen as the most vigorous and perfect of one hundred and fifty specimens. Their only defects to the eye were a frequent shortness of one or both antennæ and in a very few instances an asymmetry

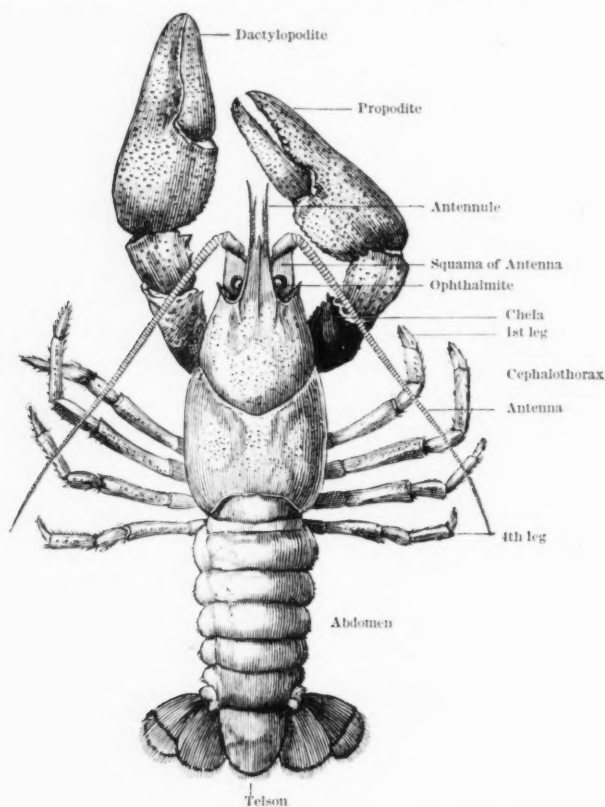


FIGURE 1.—*Astacus fluviatilis*. Tergal surface.
From Huxley: "The Crayfish."

in the size of their great claws. Each animal was at its receipt numbered on the back with white oil-paint, the females having the consecutive odd numbers up to twenty-nine and the males some of the intervening even numbers; thus each individual and its sex could be identified at a glance. They were kept in a convenient zinc tank

175 cm. long, 35 cm. wide, and 25 cm. deep, with an outlet-tube at about 15 cm. from the bottom of one end, while a stream of water from the city pipes entered at the other; when this water in the dead of winter became too cold, it was warmed by flowing through a double worm in a 25-litre copper boiler heated by a Bunsen flame. The tank was tilted longitudinally at an angle of about 10° , thus allowing a few centimetres of the bottom of the upper end to be free of water. A thin spruce board was anchored so that it would float near the upper end of the tank.

The general habits of these animals under these artificial conditions will not detain us long, although notes as to a few points may in passing be deemed of interest.

Eating and Taste.—The food most readily taken by these crayfish was meat containing much blood and juice. Frog livers and muscles and lean raw beef were often given them, and occasionally some artificial "worms" of fresh fibrin, which were greedily attacked. The third maxillipedes, serving also to press the bit of food against the jaws, seem to bear the organs of taste, for they would remain extended over a bit of meat sometimes a few seconds and then seize it and press it against the mouth, the foremost pair of feet serving to hold it firmly laterally and to tear it by pulling it sidewise back and forth from the jaws. One individual (No. XV) repeatedly grabbed a strip of thin sheet rubber cut to represent closely a leech, when this was waved through the water above her; each time it was pressed into the jaws and chewed upon actively. This whole process was done several times by her. None of the others showed the slightest interest in the object, although each had an equal chance. Many, however, similarly seized, on tip-toe, so to say, bits of red, worm-like fibrin.

Fear.—Horrification is developed to a remarkable degree in the crayfish, and offers in itself a problem as to instinct or co-ordination not easily solved at present. The phenomena themselves are well-known: Active stimulation or sometimes even the approach of an object such as would naturally give occasion for "fear" perhaps, often causes the animal to rise violently as far from the surface on which it rests as the extreme arrangement of chelæ, legs, and abdomen can bring about, all these being meanwhile in a state of extreme tonicidity amounting often almost to a cramp of the muscles. Usually also in this condition the squamæ of the antennæ are adducted to their limit, a reaction observed constantly in some individuals but in

others apparently seldom or never. The interesting point of this whole phenomenon is that, assuming with Darwin that this process was developed as a protective instinct intended to frighten away enemies (a presumption not as yet superseded), in this case teleology has outdone itself, for a passionate or excitable individual often rises so vigorously and on limbs so rigid that he falls on his back in a way to put himself most completely into the power of any foe, being then comparatively helpless and exposed. It is interesting to note that this phenomenon may be occasioned sometimes as fully by stimuli received by the animal through the eyes only as by mechanical and often repeated irritation of the body directly.

Touch. — The sense of touch seems on the whole to be the sense of greatest value to the crayfish, so far as actions can indicate. By means of setæ arranged in little clumps in general all over the body, the animal's knowledge of mechanical disturbance near him is fairly complete, although the antennæ are doubtless the organs of touch in particular. These latter are carried usually, during forward progression, raised and somewhat adducted in directions to best strike against objects lying in the animal's path. They are by far the most perfectly mobile of the crayfish's organs, the tip of each possibly describing a hemisphere. When one of the animals is about to walk in a direction other than that in which his rostrum points, the antenna of that side, often even two seconds in advance, turns in the direction to be taken. This may show one of two things, — either that the intention of the animal is very slow in being carried out, or that the stimulus which determines this action comes from without the organism and causes this — the most sensitive organ — to move first. When one crayfish approaches another the antenna of the latter meets the former, touching him some place, usually on the antenna. Moisture is probably perceived by means of the antennæ; one of the animals which had been a rather long time out of the water, while walking over a large sheet of paper having on it here and there a wet spot, pushed his antenna on the paper before him and traversed the moistened places. The antenna seems rather sensitive to electric currents in the surrounding water; these, when strong, like other abnormal stimuli cause the organs to be laid closely along the back. The antennæ do not seem sensitive to a remarkable extent to heat. Oftentimes it is necessary to touch a crayfish in order to arouse him; vision seems sometimes asleep; this is especially noticeable apparently when the animal is in water.

Sight. — The importance of sight in the crayfish seems sometimes over estimated, for it probably is of less use to the animal in his normal life than touch. The eyes are very sensitive to the passing near them of large opaque bodies, and the withdrawal-reaction is often very marked in these cases. Sudden turning-on of a glow-lamp (16 candle-power) over their tank usually caused half of the animals or more to stir to a greater or a less extent; turning the light off likewise caused movements, but in a smaller number. Phototaxis was not studied under precise conditions, but would seem not to obtain in either mode; the crayfish appeared neither to seek nor to avoid a 32 candle-power lamp immersed in the water of their tank, although those near it seemed regularly to turn, if necessary, so as to "face" it. Withdrawal of the ophthalmites into their sockets occurs only on contact with some hard object — not from any light-stimulus of an ordinary sort. Crayfish seem to recognize their fellows at a distance of from 15 cm. to 20 cm., for the attitude of defence is taken sometimes when a comer is that distance away. So far as observed, no individual noticed certain rather conspicuous glass cylinders in the tank by sight, for when their course took them that way they ran awkwardly into them and then backed off.

Several individuals were blindfolded by bending around the cephalon a bit of sheet block tin, properly shaped, and held in place by a wire extending along the middle of the back and under the first abdominal somite; an orifice was left in front for the play of the antennæ and the antennules. Crayfish No. XII wore this closely-fitting helmet a day. During the first hour or so he repeatedly tried to remove it, using for this purpose the three forward pairs of legs and feet. His actions while blindfolded were seemingly homologous to man's under like condition, as regards getting about: — the gait was slower and more uncertain; when an antenna touched any object, the animal instantly stopped for five seconds or so and then advanced in a more or less changed direction. When blindfolded, No. VI was prone to revolve, the circle being each time somewhat enlarged. This same animal (the most sexually vigorous of all the males) on one occasion grabbed No. IV (another male) in obviously sexual embrace, the latter soon escaping. This occurrence, never observed by me under normal conditions of vision, indicates that crayfish possibly can distinguish, in part at least, the sex of their fellows by sight. This is a result rather unexpected, for the sexual differences, especially from in front, are slight. The tin helmet did not interfere with the ac-

tion of the organs of smell, by which sex perhaps in part may be discriminated.

Smell. — On the sense of smell no adequate experimentation was attempted; a few tests, simply, were made on the sex individuals numbered from II to VII. Three odors only were employed, and not knowing what particular smells were disagreeable to the crustacean, those were naturally selected to which humanity usually objects. Asafœtida, ammonium valerianate, and long-decayed fibrin infusion in combination make a quite resistless olfactory stimulus. In all possible forms, singly and in combination, these were presented to the six subjects both in water and out of it, but it was at no time suggested by any act on their part that these odors were perceived by them. This fact is of course no proof that the sensation of smell was not experienced, but it is at least presumptive evidence that nothing in the nature of unpleasantness or pleasantness came within the sensorium of the animals, for these are usually correlated with appropriate movements.

Hearing. — No signs of a sense of hearing were at any time observed, although the range of auditory stimuli applied was large. Sudden pressures in the water, however, seemed plainly to be felt, and for these perhaps the crayfish otoliths are tuned; (this question is still in doubt even in the case of man).

Equilibrium. — Simple experiments were performed on three individuals (IV, XV, and XXV), to determine if they possessed organs concerned with bodily balance which might, as in man, be disturbed by rotation about a vertical line in the middle of the body. An earthenware two-kilo bowl was filled with water and the crayfish placed within it. A forceful stream of water was then applied to one rim, and the whole rotated at the speed desired. No. IV was rotated once a second for five minutes but showed no sign of vertigo or ataxia, and immediately afterwards ambled away quite as usual. Nos. XV and XXV were rotated similarly for six minutes with like negative results. This is a situation in which one would suppose they might at times find themselves in rapid brooks, and the result is suggestive. The compensatory movements of the ophthalmites were not studied.

Movements. — The gait was carefully watched on two different days. Without a series of kinetoscopic photographs a leg-order so complex is no easy problem to define, and individual variations are yet more difficult of record. It was learned, however, from the two or three

days of study that no precise formula or rhythmical co-ordination was constant either in the crayfish as an average, specific animal or in the individual from day to day or hour to hour. There seems in general to be a distinct tendency for the four pairs of legs to be moved forward successively from before backwards, the two legs of a pair more often moving together, yet often not. The foremost pair are limbs rather highly differentiated as prehensile organs, as toilet implements, and as arms bearing fingers much more nimble than the clumsy chelæ; on this account perhaps, in part, the first pair often fails to keep the "step." The second pair are used in general similarly, but much less often for prehension and then with far less skill. The third pair seem to be for the purpose of walking chiefly; on them most of the weight is borne at times; this pair are most often in unison of movement with the second pair. The fourth, or hindmost, legs are more or less like stiffened, although jointed, props by which the animal pushes himself, to some extent, along, and holds his place on inclines or against the current. These are obviously used quite as the convenience of the moment dictates, and are therefore more often out of the typical rhythm than any of the other pairs. In some cases the chelæ are used as legs in walking, oftener yet in standing, and in horriification, already noted, they serve to support and give stability to the raised body. The crayfish walks in every direction, most often forwards, but readily also sidewise, obliquely, or backwards.

One of the most noteworthy facts, apparently, in the motivity of the crayfish is the distinction which seems to obtain between the muscles of the cephalothorax and those of the abdomen as regards their innervation and relation to the proper will of the animal. While each of the limbs of the cephalothorax, possibly excepting the ophthalmites, may be said in a sense to be under control of the animal's will, what correspond to voluntary muscles in higher forms, the contractile organs of the abdomen, the large flexor in particular, seem to be almost "involuntary," acting reflexly and ordinarily from some sort of shock to the neural mechanism in some part of the body. The series of large muscular bundles which constitute this flexor abdominis work always together more or less and their simultaneous sudden contraction causes the crayfish to dart backwards in the act of swimming. This under normal conditions the animal appears never to do unless "frightened" or shocked; it may then make an indefinite number of graceful swimming jumps. That this

compound muscle may, however, at times come partially under voluntary control, will be shown later on. In general this distinction is sufficiently striking, indicating perhaps that the abdomen was developed by natural selection as a protective means of escape from dangerous foes, but that it has not as yet come under ordinary individual control. On the other hand the extensor abdominis muscle is much better within the influence of the animal's choice or immediate needs irrespective of stimulation from without, and is often of use to the individual, as in re-turning from the helpless supine position.

The accuracy with which the crayfish can direct and contract its chelæ and antennæ was a subject of experimentation until abandoned for lack of time and patience. This was because it is very difficult to induce one of these animals to make a movement which may properly be called voluntary, such as a deliberate reaching after a small object under measurable conditions. This difficulty is indeed in general an index of the psychophysical development of this species; the neural simplicity probably does not allow any action purely deliberate. When the animal is highly excited it seems as if spontaneity merged with reflex movements more than usual. It appears that when such movements with the chelæ can be induced, their accuracy is considerable, although limited by the rigidity of the hinge-joints on which the chelæ are mostly moved. The antennæ may obviously be directed with extreme nicety to any objective point. As to the quickness of these movements in relation with accuracy it is difficult to make reliable statements, the variation being so extremely large. Sometimes the swiftness with which the animal raises his claws in defence seems extraordinary and often the swimming-jump backwards follows the stimulus too quickly for ocular estimate, accuracy then being wholly disregarded. This whole field of the accuracy and speed of bodily movements in various genera up and down the animal kingdom, appears to the writer a most fertile one, especially the *development of voluntary movements* both in the individual animal and the child and in the phylogenic unfolding of every empirical grade of life.

DESCRIPTION OF THE EXPERIMENTS.

Traction strength.—The first series of experiments on the various crayfish which will be described related to their respective powers of traction. For this purpose twenty individuals were employed. Each was tested on three days, the second day two days after the first and

the third day nine days after the second. The following simple apparatus was used: A brass-wire spring having such strength that a weight of 100 grams caused an elongation of about 20 cm. was suspended from a rigid clamp about 50 cm. above a table. From the lower end of this spring a strong linen thread was passed around a large, easily-turning brass pulley and thence horizontally over a sheet of white paper to a coarse, tightly-stretched towel tacked to the table, where the thread ended in a small silk-covered wire loop of considerable size. A scale made by noting the extension of the spring when different weights were attached to the thread was then marked on the sheet of paper. The wire loop was passed around the abdomen of the crayfish at the junction between the fourth and fifth somites so as to draw from beneath, and the animal was set upon the wet towel and gently tapped with a glass rod. This invariably caused him to walk forward over the surface before him, drawing on the gauged spring. The point at which the index on the thread over the scale indicated the maximum force exerted was marked with a pen. The average time consumed by the crayfish in reaching this maximum point was from one to three minutes; fatigue then rapidly ensued. A second trial was always given immediately and sometimes a third, but it is noteworthy that the record of the second attempt was always behind that of the first by an average perhaps of fifteen per cent. This seems a good test of the general strength of the various animals, for it involves the exercise of nearly all the muscles of the larger appendages, chiefly of the ambulatory legs of course but also in a less degree of those of the chelæ and abdomen as well. Any record made by flexion of the abdomen, which occasionally occurred, was disregarded.

The accompanying Table I gives in detail the numbers derived from these tests of crustacean traction-strength. In it, the first column contains the numbers of the individual crayfish; the second their respective weights in grams; the third, fourth, and fifth columns the records of strength in grams on the three respective days of the experiments; the sixth column consists of the averages of the three performances; and the seventh the traction strength per gram of body-weight, thus reducing the results to an absolute standard, for direct comparison. The large individual average variation of these records may be seen at a glance across the columns; it is large however only from our, that is the anthropomorphic, view-point. This is a characteristic of the actions of these low forms of life, interesting

TABLE I.

Individual Numbers.	Weight of Crayfish in Grams.	Weight Pulled.			Averages. Grams.	Traction-Power per Gram of Body Weight.
		First Day. Grams.	Second Day. Grams.	Third Day. Grams.		
I	11.5	38.0	32.5	34.0	35.0	3.5
II	35.	45.0	57.0	55.0	52.0	1.5
III	45.	75.0	80.0	82.0	79.0	1.7
IV	25.	67.5	63.0	66.0	65.5	2.2
V	33.	64.5	62.5	68.5	65.0	1.9
VI	35.	66.0	67.5	49.0 *	67.0	1.9
VII	44.	98.0	102.0	100.0	100.0	2.3
VIII	28.	70.0	81.5	88.0	79.0	2.8
IX	32.	48.5	74.0	53.5	59.0	1.8
X	26.	67.5	72.5	63.0	68.0	2.5
XI	32.	64.0	99.5	89.0	84.0	2.6
XII	22.	48.0	58.0	49.0	52.0	2.4
XIII	28.	42.0	44.0	66.0	51.0	1.8
XIV	18.	44.5	58.0	59.0	57.0	3.1
XV	37.	61.0	75.5	84.0	74.0	2.0
XIX	33.	68.5	71.5	49.0	63.0	1.9
XXI	27.	72.5	78.5	62.0	71.0	2.6
XXIII	29.	58.0	64.0	60.0	61.0	2.1
XXV	29.	73.5	83.0	79.0	79.0	2.7
XXVII	19.	45.0	73.0	62.5	60.0	3.1

* One leg broken.

Average, 2.3.

to compare with the average variation of similar performances by human subjects. It is sufficient here if its relative greatness be noted. The results of the experiments will be somewhat discussed later on, when all of them may be reviewed together, and in relation to our main question.

Chelæ's pinching-strength. — The next series of observations on the crayfish with instruments of precision recorded the pinching-force of their chelæ or "claws," — in more technical terms the strength of the adductor muscle which draws the dactylopodite toward the propodite. To measure this force a myo-dynamometer describable as follows, was constructed, suited to this animal, and crabs, perhaps, alone: A piece of brass spring-wire of about $\frac{1}{16}$ inch gauge was wound twice about a small steel rod, and one end cut 20 cm., the other about 35 cm. long. The upper of these, the index arm, was bent into a depression near the double coil, thence extending straight, so that it formed with the lower portion an angle of about 40° , the two wires at the place of the depression (intended to define the place of application of the chela) being 1 cm. apart. The last 15 cm. of the lower arm was bent in the form of an arc over which the index arm was to play; on this arc was fastened a metallic scale readable to the nearest 25 grams up to 2,000 grams, and made empirically by suspending scale-weights at the notch where the chela was to pinch. Strips of "tin" were rigidly fixed to the lower arm for purposes of convenient clamping in position on the table's edge; a glass tube, lessening friction, extending along parallel to the scale to confine the index-arm and prevent its bending away, completed this simple dynamometer.

In making a test, the crayfish was taken from the tank and held near the instrument in such a position that he would seize, usually with only too great readiness, the arms of the instrument in his right chela at the notch made for the purpose. The animal was then stimulated to his most active effort by tapping with a rod about the cephalon, gently, and each subject alike. The tips of the claws of the crayfish are slightly bent inward, and it was always just within this beak that the arms of the dynamometer were taken, the pressures registered thus being the smallest exerted by any part of the forceps, these being progressively greater nearer to the hinge. After the right chela had been made to grip the instrument twice, the left was applied in a similar way, the position of each in relation to the dynamometer being constant in all respects. Indeed, in all the sets of experiments

care was used to have no partiality to any individual in any regard, thus only allowing of comparable results being obtained.

The accompanying Table II gives the numerical results of this set of experiments, although in the rather anomalous terms of body-weights. It was not convenient to mutilate each animal either dead or alive for the purpose of weighing its adductor muscles. But weighing was the only way to reduce their strength to the "absolute standard." On the other hand, the conditions of the dynamometer made it impossible to use the other common method of measuring muscular work, namely, in gram-centimetres, as there was no lifting of the maximum "weight." Of several chela muscles weighed that of the right forceps of No. V may therefore be taken as an example, although apparently a muscle stronger than the average. Its work was calculated according to the absolute standard. The mechanism is, in fine, a broad bipenniform muscle acting on a lever of the third class by means of a powerful bony tendon, thereby, as already noted, adducting the dactylopodite against the propodite. The lever acts on any resistance placed, as here, in the beak at the tip of the claw at a disadvantage in force of about nine and one half times, making this up, of course, in speed and latitude of movement. The indicated pinching-force at the tip was 1,600 grams, thus showing that the actual pull on the muscle's tendon was 15,200 grams, or 15.2 kilos. The muscle in question weighed 0.7 gram, and from this and other data it was readily calculated that the absolute strength of this particular muscle was approximately 7.6 kilos per centimetre of cross-section. This is a number somewhat less than that usually accepted for the muscular strength of man, who has the highest proportional strength. The amount by which, in general, the strength of the left forceps was smaller than the right, is very likely a rough index of the fatigue of the individual. There was no indication of any unequal usage of the two chelæ, the animal being strictly, both in form and function, in theory bilaterally symmetrical; accidents, however, make defective individuals common. In several cases, not reported in the foregoing table, the force exerted by one chela was measured while the other also was gripping an object, the animal's attention thus being divided. Under this circumstance it was found that the strength then exhibited by one chela varied between 76 and 21 per cent of that exerted when only one was in use, the average being approximately one third; this curious fact the simultaneous use of two dynamometers might have explained. The theoretic

TABLE II

Individual Numbers.	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XV	XIX	XXI	XXIII	XXV	XXVII
Weights in Grams.	35.	45.	25.	33.	35.	44.	28.	32	26.	32.	22.	28.	37.	33.	27.	29.	29.	19.
Right Chela. Grams.	1450	1600	1500	1600	1300	1550	400	1100	†	1550	1200	600	600	1425	1100	700	650	300
Left Chela. Grams.	900	1000	*	1100		1200	1400	160	380	1300	1000	675	700	1000	1250	250	*	
Right Grip in Body-wts.	41.4	35.5	60.0	48.4	37.1	35.2		34.3		48.4	54.5	21.4	16.2	43.1	40.7	24.1	22.4	15.7
Left Grip in Body-wts.	25.7	22.2		33.5		27.2	50.0	3.1	13.4	40.6	45.4	24.1	18.9	30.3	46.3	8.6		

Right chela's mean, 35.3.
Left chela's mean, 25.5

* Chela secondary and too small.

† Chela broken.

result from this set of experiments which particularly interests us here is the large individual variation, larger still than that of the traction tests just described.

Re-turning habits. — The next series of experiments involves in the subject other elements than mere physical strength, namely, individual habit and a general sort of knack. It was noticed that the individual crayfish differed considerably in their faculty of righting themselves or re-turning when they had been placed on their backs on a smooth plane level surface in water. Without assistance, there are two modes by which one of these animals may re-turn when on a sheet of glass in water of sufficient depth. The former of these, the more common method, is that which a man, for example, would employ, namely, raising himself on his limbs in a way to fall over by force of gravity; the latter, quicker and more certain by far, is by means of a quick contraction of the flexor abdominis muscle, causing a quick swimming-movement backward, which action invariably lands the animal right-side-up. On the other hand, nearly half of the twenty animals employed in this set of experiments could not within five minutes of almost constant endeavor re-turn by either or any means.

To study the nature of the various individuals' habits in this particular and their constancy, whatever they were, accurate account was kept of the performance of each on three different days, both as to the ultimate outcome of each attempt to re-turn (five minutes being the limit of endeavor) and as to the time required. A very large porcelain evaporating-dish containing water at 23° and 10 cm. deep was employed for the experiments. A stop-watch accurately gave the time in each case. The crayfish was gently placed on its back in the middle of the bottom of the dish, whereupon it invariably began struggles more or less well directed toward re-turning. Five trials of each animal's faculty were made in succession on each of the three days, unless its complete inability to turn over was obvious after one or two five-minute struggles, when sometimes the rest of the five performances were omitted, for it had been learned empirically that an individual which could not re-turn within five minutes could not do so at all, at least not within the limits of a convenient experimental period.

The completeness of the numerical results of these experiments was lessened by the unexplained death of some of the subjects between the second and the third trial-days, which were some weeks

apart. The performances of the two first days were as follows. Three of the twenty crayfish invariably re-turned by proper balancing in periods ranging between 1.2 seconds and 49 seconds, the average being about 10 seconds; seven individuals failed to re-turn within the allotted time; one invariably re-turned by the flexion method; and the remaining nine subjects sometimes failed and sometimes employed successfully one or the other of the two methods. On the third trial-day there were eleven individuals tested, and they acted in no way generally unlike the twenty of the two previous days. Here, then, will be observed a certain degree of individual habit in a complex psychophysical process, but, perhaps more noticeable than this is its opposite, the absence of constancy. The inability to re-turn was more common than any successful method, and although the conditions all the while did not vary, even this inability was inconstant.

Training.— It was next experimented whether *training* would change a re-turning habit employed by any individual or improve the faculty in those which in these last experiments could not re-turn by any method. For this purpose, advantage was taken of the fact that a tap on the sternal surface of the abdomen, especially on the first somite, or pressure on the telson, causes an immediate flexion of the abdomen and, as already explained, a consequent quick and certain re-turning. By inducing this process a number of times in succession, it was thought possibly some sort of disposition might be impressed upon the neuromuscular mechanism which would make it easier for the animal to perform the action for himself when need presented.

Five individuals were accordingly so treated and observed, to learn if such lowly animals could be trained by the method probably most productive in their grade of development. Of these, No. III, in her test performances, on the first day had re-turned by abdominal flexion once and failed to re-turn the other four times; on the second day, failed the first time and was then used for another experiment for half an hour (being often galvanized), then tried again whereupon she re-turned by flexion five times in quick succession; on the third day, she had re-turned by balancing five times successively. She was then, for her training, induced to flex her abdomen when lying prone forty times in quick succession, each time re-turning quickly. Yet on trial she failed entirely to get over within five minutes despite strenuous efforts to that end. No. IV, which had re-turned on the two days he was tested by flexion each of the five times quickly, at the training test re-turned by flexion four times speedily and then by balancing

thirty times in succession in periods ranging between 9.7 seconds and 103.7 seconds. This animal had skill at re-turning above the rest, once almost succeeding in slowly overturning longitudinally by raising himself on the tightly-flexed abdomen. No. VII in the three previous trial-days had invariably failed at re-turning. No improvement was manifest after a training of forty flexion-re-turnings with a two-hour interval between the first and second twenties. The next day she failed on trial and five flexions had no effect. No. VIII, which had re-turned in his three days of trials either by flexion or, less often, by balancing, righted himself by balancing ninety times in quick succession, the longest period required being 13.0 seconds with an average of perhaps 5.0 seconds. The times started short, became gradually longer, then shortened slightly with an increased variation until the experiments were stopped. This animal invariably turned over the same side down even against a considerable opposing inclination of the surface, the reason for this being perhaps that the chela of that side was apparently secondary and still slightly smaller than the other, a habit thus having been acquired. Lastly, No. XXV (which had always re-turned by balancing), was stimulated to re-turning by flexion fifty times in succession (about the reasonable limit), yet showed absolutely no tendency to re-turn by flexion of her own initiative immediately afterward.

Reactions to galvanism.— Knowledge as to the relative sensitivity of the different individual crayfish to galvanic electricity was the object of the series of simple experiments next to be described. As apparatus there was employed a water-filled glass dish 24 cm. in diameter and 7 cm. deep and cylindrical in shape. About the rim of this vessel from top to bottom were fixed two tin-foil electrodes opposite each other and each about 16 cm. long. Connected with these electrodes were the poles of a battery of four Daniell cells arranged in series, and connected in such a way with a key that either one, two, three, or four cells (each slightly over a volt) could be instantly thrown into the circuit, which last contained in addition a Du Bois-Reymond key, and a Pohl commutator for reversing the current. Thus the current which was driven through the water in the glass dish could be instantaneously made or broken, reversed, or varied in four degrees of strength between one and four volts tension. Strips of glass on edge extended between the extremities of the electrodes in the dish thus confining the crayfish to a channel about 14 cm. wide with the electrodes at its ends 24 cm. apart.

The same twenty individuals which were studied as to their traction-strengths were used as subjects in this set of observations. The current from one Daniell cell through the amount of water here present appears to be about a threshold stimulus to movement for the "average crayfish." That from four cells produces a muscular tonicity which prevents the noting of its specific effects, while even with a three-cells current the reactions are so strong that the uniformity in different individuals is extreme, these reactions being in general spasmodic flexions of the jointed limbs and abdomen. We shall relate the details of the various reactions to the one-cell current and to the current of double that strength, only. The crayfish during these reactions was always head-on to the electrical flow, that is, with the rostrum pointing to the anode. The movements noted are those which occurred immediately on making the circuit with the Du Bois-Reymond key.

On making the one-cell current, crayfish I reacted by adduction of the exopodite of the telson and by no other visible movement; II, vibration of the filaments of the first and second maxillipedes and flexion of the third maxillipede; III, no visible reaction; IV, flexion of third maxillipede; V, vibrations and flexion of maxillipedes; VI, flexion of maxillipedes and sometimes closing of forceps of the right or of the left front leg; VII, slight adduction of right antenna; VIII, no visible reaction; IX, X, XI, XII, ditto; XIII, vibration of antennæ; XIV, no visible reaction; XV, marked flexion of third maxillipedes and adduction of antennules; XIX, vibration of antennules; XXI, adduction and lowering of antennules; break caused opening of forceps of front leg; XXIII, slight general forward movement; XXV, no visible reaction at make; break caused relaxation of forceps of front legs; XXVII, no visible movement at make; break caused general slight movements.

The make of the two-cells current caused in I the same reaction as before plus slight rotation of right chela; II, same as before plus slight flexion of chelæ and adduction of antennæ; III, first time, slight forward starting of whole body, afterwards slight flexion of right chela; IV, slight extension of left chela, flexion of right chela, closing of forceps of same, adduction of antennæ; V, first time, general starting; afterwards, closing of forceps of front legs and slight flexion of chelæ; later on, violent flexion of abdomen; VI, same as before save that "closing of the forceps of the right or left front leg" was now constant; VII, same as before plus general movement of body;

VIII, flexion of abdomen, and of chelæ and adduction of antennules; IX, flexion of right chela; X, flexion of chelæ, closing of forceps, flexion of the third maxillipedes; at break, relaxation of forceps of front leg; XI, slight flexion of right chela and flexion of abdomen; XII, slight flexion of chelæ; XIII, no visible constant reaction; at break, relaxation of limbs; XIV, flexion of chelæ and adduction of exopodites of the telson; XV, flexion of maxillipedes and of right chela; extreme abduction of left antenna, adduction of antennules; XIX, adduction of right antenna, flexion of chelæ; XXI, slight flexion right chela, vibration of antennules; XXIII, general slight flexion of limbs; XXV, vibration of the third maxillipedes; XXVII, slow general flexion of limbs. One cell occasioned flexion of the abdomen in none of the subjects; two cells in three of them; three cells in seven of them; and four cells in eleven of them. Perhaps the wide application of this method would reveal interesting relations between stimulus and reaction.

Galvanotropism. — The next series of experimental observations related to individual variations in galvanotropism. The same twenty individual crayfish that were used in the experiments just described were studied in this regard, each of them being observed twice at least on two different days. The apparatus used was likewise that of the last set of experiments, the Pohl commutator being here of essential importance for instantly changing the anode from one side of the dish to the other. The individual reactions in this case will not be detailed because every one of the twenty crayfish invariably went to the anode even with a two-cells current if given time enough. Very often he reacted more quickly with a four-cells current, although this in some cases caused tonic contractions which impeded movement. The individual variations were of course in the two directions of required time and method, but these would be very difficult to describe succinctly; the essential point for us will be noted later on. The hibernation instinct, impelling the crayfish to remain in dark corners (in these tests especially near the cathodal electrode), seemed to oppose the galvanotropic tendency somewhat. The arrival at the anode was usually promptest when the abdominal end pointed in that direction when the current was made, the animal then jumping or swimming thither by abdominal flexion. The arrival was slower when the crayfish was head-on to the current, in which case he scrambled to the anode or walked more or less deliberately. It was least rapid when he was in a position transverse to the current lines, in which

case general movements were continued until the animal became turned partly about, when the anode-seeking became much more certain. Rarely only would the crayfish sidle to the anode directly. The sensitivity of the organism to galvanism seems to lessen after a few minutes, both the reactive movements in general and the galvanotropism decreasing. That the electricity acts as a decided irritant is shown by the frequency with which the crayfish stroked their most sensitive appendages, as for example the antennæ and maxillipedes and ophthalmites with their fore "feet" and their chelæ. Oftentimes the current appears to act as a true *motive* for seeking of the anodal pole, for an animal occasionally would go to the anode a number of seconds after the circuit had been broken, indicating possibly some more or less persistent change in the cells of some part of the neuromuscular mechanism. No. XII went back and forth to the anode, as the current was alternated, in periods as short as three seconds; but most often the reaction-time was considerably longer, requiring often several minutes, during which the continual uneasiness of the organism would gradually resolve itself into slow progress toward the anode. If the crayfish were insensitive and settled down in a corner sometimes shocks frequently alternated were necessary to arouse them. The vessel employed was obviously not the best devisable for a study of the phenomenon as such, nor was it so intended, comparison of individual reactions being here our sole aim. No. XXV tried persistently to climb out of the glass dish whenever the current was on, although she was a small animal and without record of unusual sensitivity.

Hypnosis. — As a phenomenon having a still larger mental element, perchance, in its production, the "so-called hypnosis" was next employed. Eighteen individuals — those which used the dynamometer — were compared. Whatever be its nature and explanation, the condition referred to is simple enough practically, it being merely a greater or less permanency of position obtained whenever an animal of certain psychophysical simplicity is forcibly held in that position for a time. The crayfish in general readily comes under the influence, although with very various readiness, as will be seen. Each animal was hypnotized several times, and data as regards required time, duration of hypnosis, mode of awakening, etc., carefully kept, for both of the factors first-named enter the problem of comparing the susceptibilities of various individuals to the hypnotizing force. The method employed was simple enough, the animal merely being strongly held

with both hands in the chosen position in a way to prevent all possible external movements, until he no longer struggled. Apparently the only condition as regards posture is that it should be stable in relation with gravity; otherwise the shock of falling awakened the subject when the hands of the experimenter were removed.

The times required for hypnotizing the various crayfish varied largely, and the individuals seemed to have no constancy of their own. The period of impression was rarely under a minute, one case however (XIII, third time), requiring only 31 seconds (lasting about ten minutes), while in another subject (X, second time), seven minutes failed to reduce the spontaneity in the least so far as observable. Between these extremes of time every period, so to say, was recorded, the majority being about two minutes. A 16 candle-power glow-lamp held close before the subject's eyes seemed sometimes to shorten the time required. The period during which the animal remained in the imposed posture seemed to be proportionate to the time the will-subduing force was active, except for very brief periods of impression which were too short for a fair effect to be produced. The actual periods of persistence also varied largely in these experiments, the longest observed being about 27 minutes (XXV, second trial), although the laboratory conditions were not of the best at the time for this particular purpose, there being somewhat too much noise, jarring, and variation of light. Susceptibility seems to increase distinctly with repetition of the impression on the average, but this is very erratic. Crayfish X, for example, required at his first experience two minutes for an effect lasting eleven minutes, but at the next trial seven minutes failed to impress him in the least, while at the sixth attempt five minutes of the influence similarly had no effect; a month or so later at the second trial an impression of ten seconds endured twenty-eight minutes. The hypnosis comes to a close usually with suddenness, sometimes with even a general leap when the animal has been in the head-down position. Any sort of sensory stimulation seems able to bring the condition to an end, probably excepting sound stimuli, which were never at any time successfully employed in this research. When held in either the sternal or the tergal position the effect is produced as readily as in any other perhaps, although it is less easy to produce unusual muscular and joint strains under these conditions. Under water hypnosis can also be accomplished in the case of the crayfish, but only with far greater difficulty. VI required an impression of six minutes to stay ten seconds in water, when on his back, whereas

after seven seconds' influence when out of water he stayed placed 15.5 minutes. That one may compare the actions of a crayfish hypnotized on its back and those of one merely laid on its back, the following is given: No. III, hypnotized by restraint of legs and extension of chelæ and abdomen along the table for 1.5 minutes, remained perfectly still externally for 17 minutes. Similarly placed but unhypnotized, she made lively general movements for three minutes, lay still 15 seconds, moved 45 seconds, lay still ten seconds, etc., thus continuing for some time; at the end of seven minutes she lay still a longer period, and then moved seven minutes more—when she was put back into the tank. With the production of this state unusual posture would seem to have nothing to do, for this same crayfish was suspended by the distal end of her abdomen for ten minutes in air without any sign of hypnotization, although a relatively susceptible subject.

Reaction-time.—The last formal set of experiments which we shall at this time describe were concerned with the crayfish's reaction-time, so far as he has any. Reaction-time is a psychophysical determination which in the human individual has no little importance aside from its historical interest, for it is in a degree apparently an index of the vigor of a will or personality.

The apparatus constructed in this case was as follows: No Hipp chronoscope being at hand, a rough substitute was arranged which proved to be quite sufficient for the purpose intended. Three electric pens were arranged to write on a rotating smoked drum each precisely above the other, and each was supplied by a separate storage battery of its own, the lines traced being about 3 mm. apart. The uppermost pen made a vertical mark when the signal-stimulus was applied to the crayfish, the middle pen marked the instant of the animal's reaction (closing of the forceps of one chela), and the lowest pen recorded time-intervals by means of an Ewald electrical metronome placed in the circuit. Fifths of a second were at first employed, but were afterwards changed to halves. The flexible poles of the signal circuit ended in an instrument especially constructed of sheet copper, rubber, and wood so that pressure on the point of the key bent at right angles to its handle instantly completed the current. The reaction-key was made of like materials, the poles ending in pieces of hard rubber, which being pinched together by the reactor, instantly made the current and determined the record of the time intervening since the stimulus was felt.

The method of experimentation was simply thus: A crayfish, having been blindfolded by the tin helmet already described, was placed in a rubber-lined brass clamp fashioned so as to hold him firmly but comfortably about the whole length of the cephalothorax, and at such a height above the table that his legs and chelæ could conveniently touch it. The reaction-key was then placed within either one of his usually open chelæ, and while the animal was in the ordinary state of reactive excitement or "anger" or whatever the reader may please best to think it, he was vigorously tapped with the end of the stimulation-key, most often on some part of the chela employed for reaction or else on the antennæ or elsewhere about the head. The location of the stimulation seemed to make no difference. The interval between the two marks made on the smoked paper could then be read by comparison with the accompanying time-intervals, this being the "reaction-time" desired.

Ten individuals were studied in this portion of the research, many reactions being taken from each of them. The mean value for each subject was as follows: —

II	0.20 sec.	VIII	0.23 sec.
IV	0.55 "	XI	0.30 "
V	0.25 "	XII	0.65 "
VI	0.35 "	XXI	0.33 "
VII	0.43 "	XXV	0.30 "

The mean variation, which was instructively large, will be considered later when comparing the results in general. The writer regrets that the reaction-times of more of the crayfish at first possessed are not available.

INDUCTIVE RESULTS.

Having now stated our problem and reported the greater part of the experiments performed toward its solution, we must next see what these experiments mean in that direction. Is there in an animal of an order as low as that of the crayfish anything, discoverable empirically, comparable to the psychophysical individuality of man? In such a direction lies our present inquiry.

In our set of tests of crustacean traction-strength, the most purely somatic of the functions, not strictly automatic, were investigated, for walking is in general controlled by a minimum of voluntary attention, and in these experiments the subjects only walked against resistance. Brief perusal of the table in which the numerical results of this set

of experiments are set forth shows how variable and vacillating were the performances of many of the crayfish in even this direction. No. XI, for example, on one day drew 64 grams as his maximum, and two days later 99.5 grams; XXVII, 45 grams, and at the later trial 73 grams. The smallest crayfish of the lot, No. I, drew an average of 3.5 times her own weight; while No. IX drew only 1.8 times her weight. With a mean strength of 2.3 times the body-weight, the mean variation in one case was over 50 per cent. Certain individual variations in conduct while being tested may in addition be briefly noted. Most of the subjects walked forward directly at their ordinary gait at first, but, with the increasing pressure to overcome, more slowly until, near the limit, each centimetre of advance was gained only with obviously much effort and by the putting-forward of a single leg. A few individuals acted much as does a balky horse, doubling up and refusing to proceed until left alone for some time. Others walked persistently in a curve toward one or the other edges of the table. The maximum endeavor was clearly enough defined in most cases or even in all,—there being a point reached beyond which the animal by any inducible pushing on his legs could not further advance.

In the set of experiments next described, those on the pinching-strength of the chelate forceps, there seems to be a somewhat larger "psychical" element involved than in the foregoing experiments. Here the individual showing the greatest strength gripped 60 times his body's weight, while the weakest was, as may be said, perhaps one fourth as strong, exerting a pressure equal to only 15.7 times his weight, and this was the crayfish which of all those then living made the best record at traction: 34 per cent above the mean in that test. Of two with the strongest chelæ, one was just below, and the other just above, the mean traction power. In case of the left chela, the largest mean variation recorded was about 100 per cent. In general, those which were strongest in their right chelæ made the lowest records at traction, while in case of the left chela, the correlation is more perfect, occurring, as did its tests, under a degree of fatigue. On the whole, as regards the strengths of these animals' great forceps, the results show a lack of co-ordinated constancy which would by no means be foreseen in a number of healthy crayfish living lives so nearly alike. The variation found was larger even than in the traction inquiry, and showed a much larger influence of fatigue. A not inconsiderable factor of "will-power" seemed usually present.

In the experiments on the faculty of re-turning when placed on the back in water, the crayfishes demonstrate well the fickleness or even the absence of a habit or knack which one might in advance suppose would be almost as fixed as a "reflex." In these experiments there was presented to the subject for solution a dilemma disagreeable only in so far as any unusual position is disagreeable to any animal from the disturbances which tend to arise in the visceral mechanics, from unusual strains, sensations, etc., but still so unnatural as to be in normal conditions invariably strongly opposed by immediate and almost continual attempts at re-turning to a position more habitual. This falling-over happens often enough to the animal in his natural habitat, for he is something of a climber and of necessity often falls, usually, however, among plants or rocks or roots or what-not on which he can at once lay hold and thereby right himself. When, as here, all environmental helps are lacking, there is an opportunity for an exercise of individual method. Of the twenty crayfish employed on the two different days of the experiments in question, only one re-turned each of the ten possible times by the quickest but most unnatural method, the quick flexion of the abdomen; only three turned over, within the five minutes allowed, by balancing; seven could not turn over by any method; while nine, almost half of all the individuals, sometimes turned one way, sometimes another, and sometimes not at all. This relatively large proportion of helpless individuals bears no relation to somatic strength or vigor, for those which could not re-turn were at traction and gripping at least average performers. This 45 per cent of subjects which were inconstant in method and result is what chiefly interests us theoretically—those which could not re-turn or else returned one way or another,—for the conditions meanwhile, so far as external to the animal, remained constant, and the variation must have been characteristic of their nature.

This habit-variation is all the more interesting because of the distinction already noted which seems to obtain between the movements of the appendages of the cephalothorax and the action of the large flexor of the abdomen. This is best seen in the conditions under which alone a crayfish swims, namely, never when tranquilly about his business, but only when, in the crustacean degree, frightened, or hurt. This action of the largest muscle of the crayfish is quite in line with a far-reaching theory that in general throughout the animal kingdom shocks and other disagreeable states usually are correlated

in the neuromuscular mechanism with contraction of those muscles classed as flexors.¹ The consideration, then, that so large a proportion of the individuals are uncertain in their habits of re-turning becomes still more conspicuous in view of the occasional commencement of a discrimination between voluntary and reflex control which these two empirical methods seem to imply. The only crayfish which alone invariably re-turned by abdominal flexion was the one that made the best record at gripping; this animal showed only average traction power and sensitivity. He continued to re-turn by flexion until apparently fatigue of the flexor abdominis ensued, when he adopted the balancing method, especially easy for him because one chela was smaller than the other. His obvious unusual voluntary control of his flexor muscle seems only another inconsistency in habit. Other subjects never flexed the abdomen for overturning purposes, but indefinitely struggled with legs and chelæ.

The various reactions which the constant electrical current caused the different crayfish in water to make, indicate apparently both a wide variation in the sensitivity of the various animals to this sort of stimulation and a large diversity and inconstancy in the neuromuscular apparatus or conditions, even when the external circumstances are invariable. Human beings display a like variation in sensitivity to electricity, but probably not a like diversity in their motor reactions to their sensations. No correlations apparently can be discovered between the results of this set of observations and those of the other sets. Those crayfish susceptible or insusceptible to galvanism are not in similar relations as regards galvanotropism nor hypnosis; not correspondingly quick or slow in the time of their reactions, nor weak or strong in their muscular strength. The writer has failed to discover anything in these results so far comparable to individual "temperaments" as "sensitive and weak" or "dull and strong." However useless a word this "temperament" is being proved to be, it does mean something in its psychophysical aspect of muscular strength and quickness. Even with this very limited application, evidence for its existence in crustaceans seems wholly lacking.

In the experiments on variations in galvanotropism there was an unanimity of what one may call perhaps by analogy anodotropism which is still imperfectly explained. Sensitivity to the electrical influence varied very greatly, some animals going to the anode in a

¹ See DEARBORN: The emotion of joy. Macmillan's, 1899, pp. 41 *et seq.*

second or two, as already suggested, others crawling thither along the edges of the vessel as if against their wills; some staying a long time under the cathode in the corner and others never; some going backwards by preference, slowly turning around into position for so doing, others walking deliberately forwards or sidewise. Here likewise correlations fail to appear and individual types or permanence are not at all discernible.

The sixth series of experiments described above, those on that condition which Verworn¹ thinks best to call "*die sogennante Hypnose*," gives us results far more suggestive than those just commented on in that the consequences are more pronounced. As in the other cases, these experiments were not carried out as a study of the phenomenon in question, but hypnosis was employed merely because it offered a convenient test of individual variations in a direction involving whatever of mind the lowly crayfish may properly be said to possess. However, not a little time was devoted by the writer to this interesting phenomenon in the crayfish, enough at least to show the correctness of the eminent physiologist's observations and at the same time to suggest objections to his refusal to correlate the condition with hypnosis in the human subject. These two, in short, appear to the writer to be the same process, when the necessary vast allowance is made for the difference in the psychophysical status of the man and that of the crustacean. As suggestion and still more as sensory impression (the two theories), hypnosis, broadly speaking, is but the placing of restrictions on a weaker organism by one which at the time and by means of this accepted influence is the stronger. In man this clearly is done usually by will as mind or body or both working on both of these as the will of the subject. In the crayfish, where mind is at a relative minimum, the muscles are the psychophysical side primarily impressed and the sensations, secondary, arising therefrom appear to react upon the muscles for a length of time varying largely in different cases and individuals. But this latter mode also is often applicable to human beings as well, as in certain conditions of catalepsy in which as here the muscles may be immediately controlled. Moreover, Professor J. Mark Baldwin has reported² how he restrained the reflex movements of his infant child and put her to sleep in a manner apparently quite similar to that employed in these manipulations of crayfish.

¹ VERWORN: *Die sogennante Hypnose der Thiere*. Jena, 1898.

² BALDWIN: "*Mental development in the child and the race*," 2d edn.; p. 116

In these simple experiments with hypnosis, almost more than in any others, inconstancy is supreme. It is not possible to class any of the eighteen individuals by a hard and fast criterion as either susceptible or not so. Easily and quickly hypnotized once, a crayfish often the very next trial required many times as long an impressing, and this notwithstanding an increasing susceptibility in general. Like statements apply to the duration of the hypnotic influence both in the individual and in different individuals, the variation here being found to be still more conspicuous.

The attempt to secure in case of the crayfish an individual reaction-time by the method so often employed in taking the reaction-times of human subjects, yielded results very suggestive in the direction of our general problem: on them we can, perhaps not improperly, lay much stress. The neuromuscular process in these reaction-time experiments seems to be more closely correlated with what may analogically be termed the crayfish mind than in any other of the foregoing sets of experiments. In the human subject, the quickness of the reaction seems due to the tension of the will force exerted at the moment, known as the expectant attention—a strain of waiting for the signal, the rest of the action being often subconsciously automatic. In the present case the process is somewhat different, there being here of course no precise expectation of the stimulus. Taking the place of this, however, there is a certain general excitement, directed only in a minor degree as an emotion of defence most like to anger, and which, as far as consequences are concerned, acts practically in the way anger acts in man. As respects the reaction itself, the psychophysical conditions would seem to be similar in the two animals. It would be no valid objection to our method here to say that such reactions of the crayfish are more properly reflex actions, for such do the movements of a man become practically after a few repetitions of the reaction-time process. The conditions of the experiments, especially the presence of the hard shell intervening between the stimulating or signal instrument and the muscle itself, prevented any possibility that the reaction represents a mere direct muscular excitability to stimulation. That the reaction-time of the crustaceans and even of the yet lower molluscs under certain circumstances is as short as that of the mammal, common observation shows: hermit crabs dart into their houses often with extreme quickness when frightened, and the writer remembers well the astonishing agility with which an octopus, come upon suddenly while

climbing over the rocks, was gone, — it was but a flash, and the stimulus was probably quite unexpected. It would be mere pedantry to venture to discriminate in kind these reflex reactions from those we have measured in the crayfish, yet an element of excitement or an emotion of anger was present in the crayfish while being irritated in experimentation which makes the crayfish reactions more representative of the psychophysical status of the animals. This is an element which serves as a sort of attention expectant of further stimuli, as already suggested. Thus there is here in a sense and degree a measure of the individual's "mental" and psychophysical grade.

The mean time of the reaction of the several individuals has already been given: in expressing this as a mean instead of as an average we of course have avoided the influence of extremes, yet the numbers arrived at are various enough and show a large range, namely between 0.20 and 0.65 second. Furthermore, it may be said that in several instances measurements were excluded as chance "reactions" or as possibly "anticipatory" which perhaps should have been included and others, two or three, because they seemed too long. Such a practice, quite admissible in computing human reaction-time (because the results of experience show the normal constancy of the periods), may be erroneous in dealing with animals low in the zöologic development, but to be conservative it was here employed. Were these extreme periods admitted, the range of reaction-time as studied in these experiments is for only ten crayfish rather more than a second, some of the doubtful times being approximately 0.10 second and others, although fewer perhaps, considerably over a second. But more striking even than this empirical large range among the individuals, are the individual mean variations, which are accordingly given here: —

II	0.25 sec.	VIII	0.025 sec.
IV	0.25 "	XI	0.80 "
V	0.25 "	XII	0.45 "
VI	0.35 "	XXI	0.385 "
VII	0.175 "	XXV	0.50 "

In six of these ten cases the mean variation is equal to or longer than the mean reaction-time, a fact which speaks for itself. Moreover, no correlations could be discovered between the reaction-time of an animal and his performances in the laboratory in other directions. One quick at one thing was slow or average or quick at

another apparently quite as it chanced at the moment. A slow animal does not appear to be strong or average or weak or sensitive or insensitive or clever or stupid save by "chance" combination of circumstances. These reaction-times, involving in their production as they do a psychical element larger than was involved in the other experiments, form a sort of climax to our demonstration the outcome of which, from the standard of theory, was already foreshadowed in the traction tests, most material and somatic of them all.

CONCLUSION.

The results of this tentative research being now considered in detail, only the inevitable general conclusions remain to be expressed. The *absence of correlations* and the *inconstancy* are the two significant features of the results. In a vague and purely physiological way both of these are undoubtedly present in the crustacean nature, as the numerical products of the various sets of experiments show. Such constancies and correlations are inevitable from the greater or less stability of the material "basis" of vital phenomena. It was evidence for the presence or absence in the crayfish of a less somatic sort of individuality that we sought. Of this practically nothing, save a mere evolving beginning, has been found in the crayfish. The only "individuality" of which in this particular animal evidence has been gained is a bodily individuality dependent in part rather strictly on unknown immediate internal conditions and mediately, in the long run, purely on environment.

Experiments more or less like these may in the future aid in defining the several grades of individuality in orders of animals progressively nearer to man.

ON THE ARTIFICIAL PRODUCTION OF NORMAL LARVÆ FROM THE UNFERTILIZED EGGS OF THE SEA URCHIN (ARBACIA).

By JACQUES LOEB.

[From the Hull Physiological Laboratory of the University of Chicago.]

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I. INTRODUCTORY REMARKS.

EIGHT years ago I published the results of some experiments on the effects of an increase in the concentration of sea-water upon the segmentation of the egg. I had found that the addition of a small quantity of NaCl to sea-water retarded segmentation in the egg of the sea urchin. By increasing the concentration a point was soon reached where no further segmentation occurred. If one carefully selects the minimum increase in the concentration which is able to prevent the segmentation of the egg and the eggs be kept in this solution for one or more hours, a peculiar phenomenon occurs. When put back into normal sea-water the eggs do not segment into two, four, eight cells and so on successively, but begin to divide into more than two cells very soon after being brought back into the sea-water. The longer the egg is kept in concentrated sea-water the greater is the number of cells into which it breaks up simultaneously. I repeatedly saw an undivided egg go into a morula stage within fifteen minutes after it was put back into normal sea-water. I did not make a thorough histological examination of these eggs. Dr. Conklin was kind enough to stain a lot of eggs that had been in concentrated sea-water and which showed no trace of segmentation. "Some of these eggs showed very distinctly from four to about thirty nuclei; in other eggs the segmentation of the nucleus was not so perfect.

The nucleus, extremely large, seemed to consist of several parts which, however, were still connected." These histological examinations were not thorough enough and it was my intention to have them continued. The explanation I gave for this phenomenon was as follows: "The segmentation of the protoplasm is the effect of a stimulus which the nucleus applies to the protoplasm and which makes the protoplasm close around the nucleus." On the other hand if we put an egg into sea-water whose concentration has been raised by the addition of certain salts the protoplasm loses water and this loss of water brings about a loss of irritability. There is a certain concentration at which the nucleus is still able to divide while the protoplasm loses its ability to respond to the stimuli emanating from the nuclei. This was what it seemed to me happened in the more concentrated sea-water. The nucleus divided but the protoplasm had lost its irritability on account of the loss of water. Hence there existed a segmentation of the nucleus without a segmentation of the protoplasm. But as soon as such an egg was put back into normal sea-water the protoplasm began to take up more water and respond to the stimuli of the nucleus (these stimuli I considered to be chemical). Hence the protoplasm divided at once into as many cells as there were nuclei preformed.¹

The following year Morgan stated that he had repeated my experiments and confirmed them, but was unable to agree with me as regards the nuclear division.² He found only one nucleus in the egg and concluded that no segmentation of the nucleus occurs in the concentrated sea-water but that a rapid division of the nucleus occurs when the eggs are put back into normal sea-water. As he had made only four experiments in all, I asked the late Professor Norman, who worked in my laboratory, to make a larger number of experiments in order to find out whether there was a division of the nucleus without a segmentation of the protoplasm and whether this division was mitotic. Norman found that by carefully selecting the concentration of the sea-water a division of the nucleus without a segmentation of the protoplasm occurred, and moreover that the division was mitotic.³ The number of cells into which the egg divides at once when brought back into normal sea-water is often larger than the number of the nuclei preformed in the concentrated sea-water.

¹ LOEB, J.: *Journal of morphology*, 1892, vii, p. 253.

² MORGAN, T. H.: *Anatomischer Anzeiger*, 1894, ix, p. 141.

³ NORMAN W. W.: *Archiv für Entwicklungsmechanik*, 1896, iii, p. 106.

It therefore seems as if a further division of the nuclear matter occurs immediately after the eggs are put back into normal sea-water. The addition of sodium chloride seemed to injure the eggs and I asked Mr. Norman to try the effects of other chlorides. He found that an increase in the concentration of sea-water by the addition of $MgCl_2$ is less harmful than that of any other chloride.

It seems to me that it is necessary to discriminate in these experiments between two different effects produced by the addition of salts (or the increase of the concentration of sea-water). The one effect is that produced on the nucleus and consists of a destruction (liquefaction?) of the nuclear membrane and possibly a dissolution of the substance which binds the chromosomes together. This effect seems within certain limits to increase with the concentration of the sea-water. The other effect consists in the gradual suppression of the motility of the protoplasm. This may possibly be due to a decrease in the fluidity of the protoplasm (water rigor). This effect also becomes stronger with the increase in the concentration of the sea-water. At a certain point in the increase of the concentration the nuclear membrane will be dissolved and the chromosomes scattered (through protoplasmic motions) while the protoplasm is no longer able to undergo segmentation. This was observed by Norman. If the concentration is a little higher the dissolution of the nuclear membrane occurs, but the protoplasm on account of its rigor is unable to scatter the chromosomes and to segment. If such eggs be put back into normal sea-water the protoplasm gradually loses its condition of rigor. The motions that lead to the scattering of chromosomes return sooner than the ability to segment. In such cases the process probably occurs in the form in which Morgan observed it. There may be intermediate stages and variations.

I mention these experiments, mainly for the reason that they led Morgan to a very important step, namely, to try the effect of an increase in the concentration of sea-water upon *unfertilized* eggs. He found that eggs that were put into sea-water whose concentration had been raised by the addition of $1\frac{1}{2}$ per cent NaCl or $3\frac{1}{2}$ per cent $MgCl_2$ began to segment into two or more cells when put back into normal sea-water. This segmentation went in some cases about as far as the 64-cell stage but then the development stopped.¹

Mead made the observation that the unfertilized eggs of *Chaetopterus* could be caused to throw out the polar bodies by the addition

¹ MORGAN T. H.: *Archiv für Entwicklungsmechanik*, 1899, viii, p. 448.

of a small amount of KCl to sea-water. The addition of NaCl had no such effect.¹ Last year Dr. Matthews made an experiment with rennet ferment which he did not publish. In a previous paper on the origin of fibrinogen he had expressed the idea that the origin of the astrospheres in a cell was due to a process of coagulation. He tried the effect of rennet ferment upon unfertilized eggs of the sea-urchin to see whether he could in this way cause the egg to develop. The eggs were put into a solution of rennet tablet and when taken out began to segment, but the development did not go beyond the division into a comparatively small number of cells. The phenomenon resembled the one described by Morgan to such an extent that Matthews came to the conclusion that it was not the rennet which acted in his experiments but the salts in the rennet tablets. In other words, it was practically the increase in the concentration of the sea-water which brought about the segmentation of the unfertilized egg just as in Morgan's experiment.

There are some earlier observations concerning the fact that unfertilized eggs may show the beginnings of segmentation. Hertwig mentions² that the eggs of Arthropods, Echinoderms and Annelids show a beginning of segmentation when left in sea-water for a long time (about twenty hours). Tichomirow is quoted as having produced artificially a beginning of development in the eggs of Bombyx. But these eggs are naturally parthenogenetic. Nussbaum³ has repeated these experiments, and as far as I can see the unfertilized eggs of Bombyx seem to develop naturally just as well as with the treatment given them by Tichomirow. There is a statement by Dewitz⁴ that treatment with corrosive sublimate causes the eggs of a frog to show a beginning of segmentation, but if I am not mistaken Dewitz made no sections through these eggs and he himself expressed his doubts to me as to whether there was a real segmentation or whether the surface of the egg simply resembled that of segmented eggs.

Kulagin recently made the following statement: "I exposed unfertilized eggs of fish and amphibians to diphtheria antitoxin and noticed in many the process of segmentation."⁵ As this one sen-

¹ MEAD: Lectures Delivered at Woods Hole. Boston, 1898.

² HERTWIG, O.: Die Zelle und die Gewebe, vol. i, p. 239.

³ NUSSBAUM, M.: Archiv für mikroskopische Anatomie, 1899, liii, p. 444.

⁴ DEWITZ, J.: Biologisches Centralblatt, 1887, vii, p. 93.

⁵ KULAGIN: Zoologischer Anzeiger, 1898, xxi, p. 653.

tence is all he has published about his experiments it is impossible to express an opinion concerning them. If there was a real segmentation it still remains an open question whether it was not caused by the salts of the serum. This constitutes about all the data existing at the time I started my experiments.

I had in the meantime made my experiments on the effects of ions upon the rhythmical contractions of muscle and reached the conclusion that by changing the ions contained in a tissue we can impart to it qualities which it does not ordinarily possess.¹ I concluded that it might be possible to produce blastulæ or even plutei from an unfertilized egg by merely changing the ions in the egg. Such changes were possible in three ways: first by altering the qualitative constitution of the sea-water without altering its total osmotic pressure; second, by altering its osmotic pressure by the addition of certain salts; and third by combining both methods. The last way led to positive results.

I began my experiments with a study of the effects of various ions on the *development of the fertilized egg*.

II. THE EFFECTS OF VARIOUS IONS UPON THE FERTILIZED EGGS OF ARBACIA.

The eggs were fertilized in normal sea-water and after five minutes were put into the various solutions. The greatest care was used with the eggs and as little sea-water as possible was added to the artificial solution to be tested. The eggs were collected in vessels in such a way as to form a thick layer. One or two drops from a pipette gave all the eggs needed for an experiment. These two drops consisted chiefly of eggs with the minimum amount of sea-water. The volume of each of the artificial solutions was 100 c.c.

One chloride in solution.—In a $\frac{5}{8}$ *N* NaCl solution ten, twenty and in one case fifty per cent of the eggs began to segment. They very rarely reached the 16-cell stage. The majority of the eggs stopped developing at the 2-cell stage. The size of the cells was as a rule unequal from the beginning.

In a mixture of 90 c.c. of this solution with 10 c.c. of distilled water about 80 per cent of the eggs developed, some of which even reached the 32-cell stage. In making more dilute solutions fewer

¹ LOEB: Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln hervorrufen. Festschrift für Fick. Braunschweig, 1899.

eggs segmented and in solutions that were more dilute than a mixture of 70 c.c. $\frac{5}{8}$ *N* NaCl+40 c.c. distilled water as a rule no eggs segmented. This was not due to the reduction in the osmotic pressure, for in a solution of 70 c.c. $\frac{5}{8}$ *N* NaCl+30 c.c. of cane sugar of the same osmotic pressure but very few eggs began to segment. They did not develop beyond the 4-cell stage. In equal parts of the NaCl and the sugar solution not an egg segmented.

In a $\frac{5}{8}$ *N* KCl solution about 70 to 80 per cent of the eggs segmented and many reached the 8-cell stage. A slight dilution of the KCl allowed the eggs to reach the 32-cell stage. Even in a mixture of 60 c.c. KCl and 40 c.c. distilled water about five per cent of the eggs began to segment but only reached the 2- or 4-cell stage. In more dilute solutions no segmentation occurred. The cleavage cells were more equal in size than in the NaCl solutions. It is obvious that a pure KCl solution is more favorable for segmentation than the pure equimolecular NaCl solutions. In a former paper I published a similar observation on the *Fundulus* egg.

In a $\frac{1}{8}$ *N* MgCl_2 solution the eggs reached the 32-cell stage and in more diluted solutions, for instance 50 c.c. MgCl_2 +50 c.c. of distilled water the development went even further (64 cells or more). But the various experiments with a pure MgCl_2 solution varied somewhat in their results. On the whole the MgCl_2 was more favorable than the KCl or NaCl.

In a $\frac{1}{8}$ *N* CaCl_2 solution there was at the best only the beginning of a segmentation. In a mixture of 90 c.c. $\frac{1}{8}$ *N* CaCl_2 with 10 c.c. of distilled water I occasionally saw an egg in the 2-cell stage. In more dilute CaCl_2 solutions no trace of a segmentation occurred. Hence Mg and K were more favorable than Na- and Ca-ions for the concentration used in the experiments. It is very evident from these experiments that the optimum concentration for each of these four chlorides is different.

In a $\frac{5}{8}$ *N* LiCl solution the majority of eggs remained unsegmented and only very few reached the 2-cell stage. Mixtures of LiCl with sugar were no more advantageous. In pure glycerine and sugar solutions of the same osmotic pressure as that of a $\frac{5}{8}$ *N* NaCl solution no egg segmented. It is evident that the quality of the ions is of more importance in these experiments than the osmotic pressure and that NaCl is not an indifferent substance.

Two chlorides in solution.— In a solution of one chloride the eggs of *Arbacia* cannot reach the blastula stage. Are mixtures of two

chlorides more favorable for segmentation? Among the possible mixtures of the two chloride solutions of the same osmotic pressure as the sea-water I found those between $\frac{1}{8}^0$ *N* MgCl_2 and $\frac{1}{8}^0$ *N* CaCl_2 the most favorable. The following twelve mixtures were prepared:¹—

1.	100 c.c. $\frac{1}{8}^0$ <i>N</i> MgCl_2 +	0 c.c. $\frac{1}{8}^0$ <i>N</i> CaCl_2
2.	95 " " +	5 " "
3.	90 " " +	10 " "
4.	80 " " +	20 " "
5.	70 " " +	30 " "
6.	60 " " +	40 " "
7.	50 " " +	50 " "
8.	40 " " +	60 " "
9.	30 " " +	70 " "
10.	20 " " +	80 " "
11.	10 " " +	90 " "
12.	0 " " +	100 " "

In the first solution the eggs reached the 32-cell stage. In the second, third, and fourth solution they formed blastulæ, which however did not move. I first thought that for the motility of the cilia the presence of other ions might be required, but I found that blastulæ that had developed in normal sea-water continued their motion for two days in a solution of 80 c.c. of $\frac{1}{8}^0$ *N* MgCl_2 + 20 c.c. of sea-water. It is possible, however, that in such a solution cilia cannot be formed. I placed a lot of these eggs that had reached the blastula stage in a mixture of MgCl_2 and CaCl_2 in normal sea-water. The next morning they moved about in the most lively manner. It is certainly contrary to the current ideas concerning adaptation that the egg of *Arbacia* should reach the blastula stage in a solution which is practically free from Na-ions.

In the fifth solution only very few eggs segmented and reached the 8-cell stage while the other solutions were still worse. The segmentation was more regular the more Mg the solution contained and became more irregular the more the Ca-ions predominated. One of the chief features of this irregularity was the unequal size of the cleavage cells. As in certain eggs the unequal size of the cleavage cells is a characteristic feature which plays a great rôle in the theories of development, it is of interest that such differences can be brought about through the presence of a certain quantity of definite ions, especially of Ca- and Na-ions.

¹ It will save repeating these figures if I may state here that the same twelve proportions were used in all the following experiments with two chlorides in solution.

In the mixtures of $\frac{5}{8} n$ NaCl with $\frac{1}{8} n$ MgCl₂ the results were not so good. No swimming blastulæ were formed. In solutions of 90 to 30 c.c. of MgCl₂ with 10 to 70 c.c. of NaCl a morula stage was reached.

Mixtures of $\frac{1}{8} n$ MgCl₂ with $\frac{5}{8} n$ KCl were still less favorable. The solutions with more MgCl₂ than KCl reached the 32-cell stage or even went a little further in their development.

Mixtures of $\frac{5}{8} n$ KCl with $\frac{1}{8} n$ CaCl₂ allowed only the beginning of the segmentation and this only as long as CaCl₂ was used in very small quantities. In 96 c.c. KCl + 4 c.c. CaCl₂ one egg in a thousand went into the 2-cell stage or formed two large cells with two micromeres, but in 90 c.c. KCl + 10 c.c. CaCl₂ not one egg segmented and the solutions with more Ca were not more favorable.

A combination of $\frac{5}{8} n$ NaCl with $\frac{1}{8} n$ CaCl₂ was equally poisonous. Even in 98 c.c. NaCl + 2 c.c. CaCl₂ the eggs did not go beyond the beginning of the segmentation, and in 96 c.c. NaCl + 4 c.c. CaCl₂ the eggs died in the 4-cell stage. It is worthy of mention that in these solutions the cleavage cells were very unequal in size.

Mixtures of $\frac{5}{8} n$ KCl and $\frac{5}{8} n$ NaCl were on the other hand almost as favorable as the MgCl₂ solutions. In 98 c.c. NaCl + 2 c.c. KCl the eggs reached the 64-cell stage or went even beyond this. It was the same in 96 c.c. NaCl + 4 c.c. KCl for almost every egg divided. With more KCl and less NaCl the results were less favorable. In a former paper we pointed out that the comparative harmlessness of K-ions for the phenomena of cell division is in striking contrast with the harmfulness of the same ions for the phenomena of muscular contraction. We thus see that the following two combinations of two chlorides in solution are the most favorable for development: (1) 90 c.c. $\frac{1}{8} n$ MgCl₂ + 10 c.c. $\frac{1}{8} n$ CaCl₂; and (2) 98 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{5}{8} n$ KCl.

Three chlorides in solution.—Neither with one nor with two chlorides in solution was it possible to obtain swimming blastulæ. From the experience with *Fundulus*¹ I expected that a combination of three metal ions (especially NaCl, with small amounts of KCl and CaCl₂) would allow the eggs of the sea-urchin to complete their development. This was indeed the case. In a mixture of 96 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{5}{8} n$ CaCl₂ + 2 c.c. $\frac{5}{8} n$ KCl the eggs not only reached the blastula stage and swam around in the most lively way, but they reached the gastrula and even pluteus stage, with the exception, however, that

¹ LOEB, J.: This journal, 1900, iii, p. 383.

practically no skeleton was formed. Such larvæ lived for about ten days in this solution!

We might think that the NaCl is an indifferent substance, and that the Ca- and K- ions are responsible for the effect. From what has been shown in the foregoing papers of this series it follows that this assumption is erroneous. The same can be proved again directly for this case. I had cane sugar and glycerine solutions prepared of the same osmotic pressure as a $\frac{5}{8} n$ NaCl solution. Table I gives the results of a series of experiments.

TABLE I.

	Character of solution.	How far the eggs developed.
1	96 c.c. $\frac{5}{8} n$ NaCl + 2 $\frac{5}{8} n$ KCl + 2 $\frac{1}{8} n$ CaCl ₂	Pluteus (without skeleton).
2	96 c.c. $\frac{1}{5} n$ MgCl ₂ + " + "	Unsegmented.
3	96 c.c. $\frac{5}{8} n$ LiCl + " + "	Mostly unsegmented, few reach the 2-cell stage.
4	96 c.c. cane sugar + " + "	Unsegmented.
5	96 c.c. glycerine + " + "	Unsegmented.

The results could not be more striking. MgCl₂ is more favorable for the segmenting egg than NaCl, but still with the addition of Ca- and K- ions not an egg segments! These experiments prove once more that the conception formed in the previous papers is correct, namely, that a pure NaCl solution is poisonous, and that it requires a small amount of both Ca- and K- ions to antagonize the poisonous effect of a NaCl solution. It seems that for the egg of the sea-urchin the three metal ions in the above mentioned proportion give the colloids those physical properties which allow them to go through the changes of cell division and assimilation required for the process of development. I next tried whether it was necessary that the Ca- and K- ions be added in equal proportions to the NaCl. Table II gives the results of such experiments.

It is obvious that the proportion of K- and Ca- ions may vary within certain limits as long as they are present in sufficient quantity. I will add that I have not yet found any other combination of three chlorides that yields swimming blastulæ. In the foregoing papers I mentioned that the anions are not indifferent, and that in a NaBr solution the rhythmical contractions of a muscle begin even sooner than

in an equimolecular NaCl solution. I made some experiments on the effect of bromides on development. *In a solution of 96 c.c. $\frac{5}{8}$ n NaBr + 2 c.c. $\frac{5}{8}$ n KCl + 2 c.c. $\frac{1}{8}$ n KCl the eggs developed into normal blastulæ.* In a solution of 96 cc. LiBr + 2 cc. CaCl₂ + 2 cc. KCl the eggs reached the 16-cell stage, while in the corresponding LiCl solution practically no segmentation took place. All these experi-

TABLE II.

	Nature of the solution used.					Stage of development reached.
1	96 c.c. $\frac{5}{8}$ n	+ 2 c.c. $\frac{5}{8}$ n KCl	+ 2 c.c. $\frac{1}{8}$ n CaCl ₂			Normal blastula.
2	"	+ 2 "	+ 1 "	"	"	"
3	"	+ 2 "	+ $\frac{1}{2}$ "	"	"	"
4	"	+ 1 "	+ 2 "	"	"	"
5	"	+ $\frac{1}{2}$ "	+ 2 "	"	"	"
6	"	+ 1 "	+ 1 "	"	"	Normal blastula developed a little more slowly.
7	"	+ $\frac{1}{2}$ "	+ $\frac{1}{2}$ "	"	"	No blastulæ. Stopped at 8-32-cell stage.

ments together give us the impression that different combinations of ions may exist which all have the same effect. *It seems as if the physical condition of the colloids were the essential point, and that this might be affected by various ion combinations in the same way.*

Solutions which allow the formation of a skeleton. — The next question was what ions should be added to the above mentioned solutions in order to obtain plutei with a skeleton. I found that a trace of Na₂CO₃ has that effect. In a solution of 95 c.c. $\frac{5}{8}$ n NaCl + 2 c.c. $\frac{1}{8}$ n CaCl₂ + 1 c.c. $\frac{5}{8}$ n KCl + 1 c.c. $\frac{1}{8}$ n NaCO₃ a skeleton was formed within three days. This skeleton was not quite normal. It showed a formation of knobs and spheres which I never saw in the skeleton formed in normal sea-water (Fig. 1 B). I was anxious to obtain plutei with normal skeleton, and succeeded in doing so by adding a trace of MgCl₂ to the above mentioned solution. The solution which yielded plutei with a normal skeleton consisted of 95 c.c. $\frac{5}{8}$ n NaCl + 1 c.c. $\frac{1}{8}$ n MgCl₂ + 1 c.c. $\frac{5}{8}$ n KCl + 2 c.c. $\frac{1}{8}$ n CaCl₂ + 1 c.c. $\frac{1}{8}$ n Na₂CO₃. The skeleton is sketched in Fig. 1 A. It was therefore evident that a small amount of Na₂CO₃ allowed the formation of a

skeleton. The addition of Na_2CO_3 causes an addition of HO -ions as well as of CO_3 -ions. Which of the two was responsible for the formation of a skeleton? *The substitution of KHO for the Na_2CO_3 did not allow the formation of a skeleton. We must therefore conclude that it is the CO_3 -ion which is essential.*¹

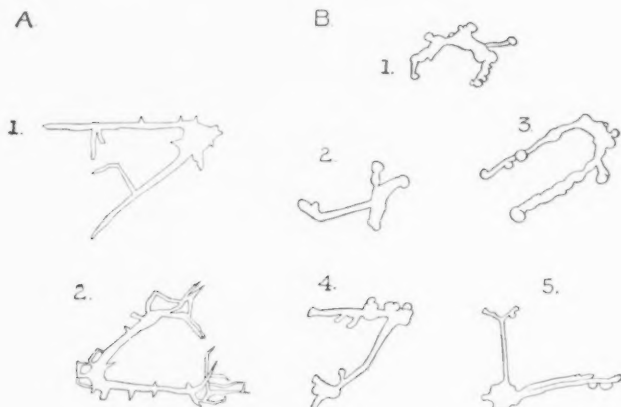


FIGURE 1. A. Normal skeleton formed in a mixture of 95 c.c. $\frac{5}{8} n$ NaCl + 1 c.c. $\frac{5}{8} n$ KCl + 2 c.c. $\frac{1}{8} n$ CaCl_2 + 1 c.c. $\frac{1}{8} n$ Na_2CO_3 + 1 c.c. $\frac{1}{8} n$ MgCl_2 .
B. Abnormal skeletons formed in a mixture like the one above, but without MgCl_2 .

Conclusions. — We thus see that a mixture of 95 c.c. $\frac{5}{8} n$ NaCl (or NaBr) + 2 c.c. $\frac{5}{8} n$ KCl + 2 c.c. $\frac{1}{8} n$ CaCl is sufficient to allow the fertilized egg of *Arbacia* to develop into the gastrula stage. But does this force us to conclude that the 3 kations Na , K , and Ca are utilized by the egg for the process of development? I think that our previous experiments on *Fundulus*² may serve as a criterion in answering the question. In a pure NaCl solution the young fish died rapidly, while in the above mentioned mixture they remained alive. And yet this same fish could live indefinitely in distilled water. This proves that it does not require any ions from the surrounding medium. It might be possible that only Na -ions were needed for the development of the sea-urchin egg. In this case the K - and Ca -ions would

¹ For further facts concerning the formation of a skeleton in sea-urchins see HERBST: *Archiv für Entwicklungsmechanik*, 1896, ii, p. 455; and DRIESCH: *Ibid.*, 1899, ix, p. 137.

² LOEB: This journal, 1900, iii, p. 383.

have to be present in order to prevent the poisonous effects which a pure NaCl solution would produce. On the other hand we found that in a mixture of $MgCl_2$ and $CaCl_2$ which is practically free from Na-ions the eggs can develop and almost reach the blastula stage. This makes it still more difficult to state positively that the Na-ions of the surrounding medium are needed for the development of the sea-urchin egg. Perhaps it is safest to assume that for the process of cell division and development a certain physical condition—a certain labile equilibrium—of the protoplasm or the colloids has to be maintained. This requires certain ions in definite proportions, either Na, K, and Ca (or other combinations, for instance Mg and Ca). Distilled water is a poison for the eggs of *Arbacia*. Hence, if Na be one of the ions of the surrounding solution, Ca- and K- ions are likewise required.

My results differ somewhat from those obtained by Herbst.¹ But I pointed out in my last paper that Herbst was misled by his method.² This method consisted in making a solution of the same complication as the sea-water, in which however one constituent was omitted. In case the eggs did not develop in such a solution, this was considered a proof that the constituent omitted was necessary for the development of the eggs. My experiments show that this conclusion is not correct. It is quite possible that the substance which was omitted or removed was not directly necessary for the egg, but only indirectly, inasmuch as it served to counteract the poisonous effects of another constituent of the solution.

It seems to me that my experiments necessitate the introduction of a new conception, namely, that of *physiologically balanced salt solutions*. By this I mean salt solutions which contain such ions and in such proportions as to completely annihilate the poisonous effects which each constituent would have if it were alone in solution. Sea-water and blood (and approximately a mixture of 96 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{1}{8} n$ $CaCl_2$ + 2 c.c. $\frac{5}{8} n$ KCl) are physiologically balanced salt solutions.

It will be necessary to investigate how far the conclusions of pharmacologists, botanists, and bacteriologists concerning the effects of various salts require a correction on the basis of these new facts and conceptions. Their consideration might even prove of use in problems of immunity and adaptation.

¹ HERBST: Archiv für Entwicklungsmechanik, 1897, v, p. 649.

² LOEB: This journal, 1900, iii, p. 383.

III. IS IT POSSIBLE TO PRODUCE BLASTULE FROM UNFERTILIZED EGGS WITHOUT RAISING THE CONCENTRATION OF THE SEA-WATER?

The early experiments with which I started had indicated that an increase in the concentration of the sea-water caused the segmentation of the nucleus in the fertilized egg. Morgan's experiments had shown that the same influence may cause the unfertilized egg to show a beginning of segmentation. In Mead's experiments, however, there was practically no increase in the osmotic pressure of the sea-water, while the nature of the ions seemed to determine the result. I wished to find out whether a blastula could be secured from an unfertilized egg without raising the concentration (the total osmotic pressure) of the sea-water. *All my experiments thus far have yielded the result that this is impossible, and that by this method only a beginning of a segmentation can be produced in an unfertilized egg.* It goes without saying that in these experiments bacteriological precautions are necessary to guard against the possibility of the introduction of spermatozoa by the instruments or of their presence in the sea-water. One has a pretty reliable criterion for the entrance of a spermatozoon into the egg of *Arbacia* in the formation of the egg membrane. An unfertilized egg has no distinct membrane, but immediately after the fertilization a very distinct and rather thick membrane is formed. As none of the eggs in the following experiment formed a membrane or showed more than the beginning of a segmentation, we may reserve the discussion of our methods of protection against fertilization for the next chapter. Unfertilized eggs of the same female were divided into three lots. One was put into a solution of 96 c.c. $\frac{5}{8}$ *n* NaCl + 2 c.c. $\frac{5}{8}$ *n* KCl + 2 c.c. $\frac{1}{8}$ *n* CaCl₂. After three and a half hours a few of the eggs showed a beginning of segmentation. After eight hours the majority of the eggs had divided into from 2 to 4 cells. Some had even gone a little further. But then the segmentation stopped. No egg had a membrane. A second lot was put into normal sea-water. Eight hours later none of these eggs had segmented or had a membrane. This indicates that a mere change in the constitution of the sea-water without any change in the osmotic pressure may cause the beginning of a segmentation of the egg. A third lot was put into a solution of 96 c.c. $\frac{1}{8}$ *n* MgCl₂ +

2 c.c. $\frac{1}{8} n$ CaCl_2 + 2 c.c. $\frac{5}{8} n$ KCl . No egg segmented. One lot of these eggs was put back into normal sea-water five hours later. A few eggs now went into the 2-cell stage, but developed no further.

In another experiment unfertilized eggs were put into a mixture of 96 c.c. $\frac{5}{8} n$ NaCl + 2 c.c. $\frac{1}{8} n$ CaCl_2 + 2 c.c. $\frac{5}{8} n$ KCl . After four and a half hours no egg segmented. They were then put back into normal sea-water, and within ten minutes one egg in a thousand divided into from 4 to 6 cells, but then the development stopped. No egg had a membrane.

In a mixture of 75 c.c. $\frac{1}{8} n$ MgCl_2 with 25 c.c. distilled water a small number of unfertilized eggs segmented. In equal parts of $\frac{1}{8} n$ MgCl_2 and $\frac{5}{8} n$ NaCl no egg segmented.

In Mead's experiments it was KCl that caused the eggs of *Chaetopterus* to throw out their polar bodies. I put unfertilized eggs of *Arbacia* first into sea-water for five hours. No egg showed a trace of beginning segmentation. After this the eggs were put for two hours into a mixture of 90 c.c. sea-water and 10 c.c. $\frac{5}{8} n$ KCl . When put back into normal sea-water, in fifteen minutes almost every other egg began to divide, but the segmentation never went beyond the 16-cell stage at the best. Neither these nor the above mentioned experiments gave constant results. The greatest differences existed in the proportion of eggs that showed a beginning of segmentation. In a former paper I had proved that the addition of a small amount of $\frac{1}{10} n$ NaHO caused an increase in the rate of development and growth of the unfertilized *Arbacia* egg, while the equally small addition of an acid (HCl) produced the opposite effect. This summer I tried the effect of HO- and H- ions upon the unfertilized egg. The following solutions were prepared:—

- | | | |
|----|-------------------|--|
| 1. | 99 c.c. sea-water | — 1 c.c. $\frac{1}{10} n$ KHO |
| 2. | 98 " | — 2 " |
| 3. | 99 " | — 1 c.c. $\frac{1}{10} n$ HCl |
| 4. | 98 " | — 2 " |
| 5. | 97 " | — 3 " |

In 1 almost every egg was in segmentation five hours later, but the segmentation was very irregular and often incomplete, and the egg showed very lively amœboid motions. Never more than 10 cells were formed. In 2 the effects were similar, but fewer eggs segmented. The segmentation did not go any further. In solutions 3, 4, and 5 not an egg showed any trace of segmentation, nor did any egg in normal sea-water segment.

Some of the eggs that were put into solutions 4 and 5 were left there only ten minutes, and then brought back into normal sea-water. Five hours later many of these eggs had begun to segment. The segmentation did not go beyond the first cell division. It should be said that the sea-water naturally contains some free HO-ions. After a short treatment with acid the HO-ions in the sea-water were able to produce an effect which they could not have had if the acid treatment had not been applied.

None of these experiments, however, led to the formation of a blastula, nor did they offer any promise of the possibility of producing blastulae in an artificial way. The experiments were made at various periods of the spawning season. After these and some other unpromising attempts I tried whether an increase in the concentration of sea-water would yield better results than a mere change in the proportion of the ions. Instead of using $\frac{5}{8}$ *n* NaCl and KCl and $\frac{1}{8}$ *n* CaCl_2 and MgCl_2 solutions I now tried $\frac{1}{8}$ *n* NaCl and KCl and $\frac{2}{8}$ *n* MgCl_2 and CaCl_2 solutions. I do not wish to give an account of all the experiments I made in this direction, but prefer to confine myself to an account of one experiment which led me in the right direction. In this experiment the following four mixtures were used:—

1. 60 c.c. $\frac{2}{8}$ *n* MgCl_2 + 40 c.c. of sea-water
2. 60 c.c. $\frac{1}{8}$ *n* KCl + “
3. 60 c.c. $\frac{1}{8}$ *n* NaCl + “
4. 60 c.c. $\frac{2}{8}$ *n* CaCl_2 + “

A lot of unfertilized eggs were distributed in these four solutions, and remained in the same for one hour and fifty minutes. They were then brought back into normal sea-water. The eggs that had been in the first (MgCl_2) solution began to show an amoeboid change of form (indicative of a segmentation) in about fifteen minutes after they were brought back into normal sea-water. About one in a thousand eggs were in this amoeboid stage. One does not see such changes in the normal egg where the membrane limits the amoeboid motions. Fifty minutes later one in a thousand eggs divided into 2 or 3 cells. The cells were of about equal size. About two and a half hours after the eggs had been put back into normal sea-water about one egg in five hundred had segmented, and the segmentation had proceeded to the 8-cell stage, although not all the eggs had reached this stage. But then the development stopped. The next morning the eggs were still without any membrane. All looked normal and healthy. In this experiment the volume of normal sea-

water into which the eggs were put after they had been in the $MgCl_2$ solution was small (only a watch-glass full). Hence the few drops of the $MgCl_2$ solution which were transferred with the eggs modified the sea-water, and I think interfered with their development. It seemed to me that by avoiding this source of error and by using large dishes with several hundred cubic centimetres of sea-water instead of the watch-glass, it might perhaps be possible to see the eggs develop further.

The eggs that had been in solution 2 (60 c.c. KCl + 40 c.c. sea-water) also began to segment. The next morning about one per cent of the eggs were divided into from 2 to 4 cells. They were all without membranes, but they looked less normal than the $MgCl_2$ eggs and soon began to disintegrate.

The third lot of eggs had been in 60 c.c. $NaCl$ + 40 c.c. sea-water. Practically none of these eggs segmented during the next twenty-four hours, and none formed a membrane. The fourth lot of eggs had been in 60 c.c. $CaCl_2$ + 40 c.c. sea-water. A few of these showed a beginning of segmentation, but every egg had a membrane. I have found since that in pure $CaCl_2$ solutions of even lower concentration unfertilized eggs form a membrane. It is possible that the formation of a membrane consists in a process of coagulation which is favored by Ca -ions.¹

I made a parallel series of experiments with fertilized eggs of the same female. The eggs were, as usual, fertilized in normal sea-water, and five minutes later were put into the various solutions. The eggs were divided into four lots, and put into solutions of the same character as in the above mentioned experiment with unfertilized eggs. Like the unfertilized eggs, the fertilized eggs remained in the solution one hour and fifty minutes. When brought back into normal sea-water those that had been in solution 1 (60 c.c. $MgCl_2$ + 40 c.c. sea-water) began to divide in fifteen minutes. The segmentation was very regular. Two hours and forty-five minutes later every egg was segmented into from 8 to 32 cells. Every egg had a membrane. The next morning a large number of eggs swam about in the blastula stage, still having a membrane. This observation is of importance, as it shows that even in eggs that were in a

¹ Hertwig showed that unfertilized eggs form a membrane in water saturated with chloroform. Herbst found that benzol, toluol, and xylol bring about the same effect. All these media have a coagulating effect. HERBST, C.: *Biologisches Centralblatt*, 1892, xiii, p. 14.

mixture of 60 c.c. $\frac{2}{8} n$ $MgCl_2$ with 40 c.c. sea-water the existence of a membrane may serve to show whether eggs were fertilized or not. The fertilized eggs that were put into the second solution (KCl) did not reach the blastula stage. They stopped at about the 32-64-cell stage. Those that had been in the third solution (NaCl) were in about the same condition, with the exception perhaps that at first the segmentation was more unequal. In the fourth solution ($CaCl_2$) no egg segmented, and only one egg in a thousand showed a beginning of segmentation, consisting of an incision at one side of the egg.

I finally wished to know how fertilized and unfertilized eggs behaved if left for eighteen hours in a mixture of 60 c.c. $\frac{2}{8} n$ $MgCl_2$ + 40 c.c. sea-water. *The unfertilized eggs formed no membrane, but a very large part, more than fifty per cent of the eggs, was divided into from 2 to 8 cells.* The fertilized eggs had a membrane. In regard to segmentation there was little difference between the two lots. It was especially this circumstance which made me hope that with a little more care it would be possible to raise living larvæ from unfertilized eggs by treating them with a suitable mixture of $\frac{2}{8} n$ $MgCl_2$ solution and sea-water.

In these and other similar experiments, which I will not describe, it was moreover evident that after the treatment with Mg-ions the character of the segmentation was much more normal than after the treatment with K- and Na- or Ca-ions. The K-ions were nearest the Mg-ions in their effect. The Ca-ions were the most unfavorable. The former experiments of Norman had also yielded the result that the Mg-ions were the most harmless for the segmentation of the sea-urchin's egg.

IV. THE ARTIFICIAL PRODUCTION OF NORMAL LARVÆ (PLUTEI) FROM THE UNFERTILIZED EGG OF THE SEA-URCHIN.

The most serious danger in experiments with unfertilized eggs is the possibility that the sea-water or the instruments contain spermatozoa. It is imperative to guard against both possibilities. The sea-urchins have practically died out in the immediate neighborhood of the Woods Hole laboratory, and we have to send out the steam launch to collect them. For this reason even at the height of the spawning season there is little danger of the sea-water containing spermatozoa in such quantities as to interfere with experiments on unfertilized

eggs. Moreover the danger that the spermatozoa contained in the sea-water of the laboratory may interfere with experiments on unfertilized eggs is not very great, even at the height of the breeding season. This is shown indirectly by the fact that in the experiments described in the previous chapter not a single egg was fertilized through contamination of the sea-water with spermatozoa. The spermatozoa if scattered in sea-water soon lose the power of impregnating the egg. Gemmill found experimentally that this occurs in less than five hours after the spermatozoa leave the testicle.¹ My experiments were carried on after the breeding season was practically over, in September, when the majority of sea-urchins contained practically no more eggs. I had already made up my mind that my further experiments would have to be postponed a year, when through the kindness of Professor Bumpus of the Fish Commission I obtained a few dozen sea-urchins that he had collected early in the season and kept in a small pond. It happened that almost every one of these animals was a female and full of eggs. Though there was little possibility that the running water of the marine laboratory could contain any spermatozoa of sea-urchins which were able to fertilize eggs, I had no right to take anything for granted in this direction. I therefore conducted with each experiment a series of control experiments to guard against the possibility of contamination of the sea-water with spermatozoa. As a rule, I proceeded in the following way. The unfertilized eggs of one female were divided into three or more lots. One lot was put into the artificial solution by which I hoped to cause the development of the unfertilized eggs. The second lot was put into normal sea-water to serve as a test or control for the presence of spermatozoa in the sea-water. The third lot was put into an artificial solution which as a rule differed less from the normal sea-water than the solution which caused the development of the egg. Whenever the eggs of one lot were put back into normal sea-water the eggs of the other lots were put into the same sea-water. Thus all three lots of eggs were kept in sea-water of exactly the same degree of contamination. In no case did a single egg of the three lots form a membrane. No egg of lot 2, which remained in normal sea-water all the time and served as a test for the presence of spermatozoa, showed any development except a beginning of segmentation (2-3 cells) after about twenty hours. In no case did any of the eggs of lot 2 or 3 develop into a blastula.

¹ GEMMILL, J. F.: *Journal of anatomy and physiology*, 1900, xxxiv, p. 163.

The chief sources of infection in such experiments are the instruments and the hands of the experimenter if he opens male and female animals at the same time. The dishes in my experiments were cleaned with fresh water, in most cases the evening before the experiment was made. The instruments which were used had been cleaned in fresh water and kept dry for twenty-four hours. In case the first animal opened was a male the instruments were laid aside, the hands disinfected, and new instruments used for the next animal. It happened that in almost every one of the following experiments the first animal I opened was a female, and thus the chief danger of contamination by spermatozoa was naturally avoided.

But even if the experiments had not been carried on with such precautions, the results obtained were of such a character as to absolutely exclude in themselves any idea of contamination by spermatozoa. In all the successful experiments the cultures of unfertilized eggs that had been treated with the right MgCl_2 solution were teeming with blastulae the next day. Twenty per cent, in some cases even more, almost fifty per cent of the eggs, had developed. In former experiments with unfertilized eggs where no such precautions were taken, I never noticed that more than perhaps one egg in a thousand developed. I shall describe each series of experiments independently. The MgCl_2 used in these experiments was chemically pure, but had been dried by heating it.

First series.—Unfertilized eggs of the same female were divided into four lots and distributed into the following four solutions:—

1. 60 c.c. $\frac{2}{8}$ MgCl_2 + 40 sea-water.
2. 100 c.c. $\frac{1}{8}$ MgCl_2 .
3. 100 c.c. $\frac{1}{8}$ CaCl_2 .
4. 100 c.c. normal sea-water.

After the eggs had been in these solutions one and a half hours they were carefully examined. Not one had a membrane and not one was segmented. Twenty minutes later one part of the eggs of each of these four solutions was transferred back into normal sea-water. The latter was the same for all four solutions. This time I took special care to see that each lot of eggs was given enough normal sea-water (about 200 c.c.). After they had been back in the normal sea-water for about two hours and fifty minutes, they were examined again. The eggs that had been in solution 1 (60 c.c. $\frac{2}{8}$ MgCl_2 + 40 c.c. sea-water) were all without a membrane. About twenty per cent of the eggs were segmented into as many as 32 cells. The eggs that

had been in solution 2 (100 c.c. $\frac{1}{8}$ n $MgCl_2$) were without any membrane and unsegmented. Many of those that had been in solution 3 (100 c.c. $\frac{1}{8}$ n $CaCl_2$) had membranes. A few were segmented very irregularly into 2 to 3 cells. All the eggs were examined again three hours later. Those that had been in solution 1 were now in a morula stage. As they had no membranes, their outline was very irregular, and I wondered what kind of blastula would result if these eggs ever reached that stage. The eggs that had been in solution 2 (100 c.c. $\frac{1}{8}$ n $MgCl_2$) were without membranes and unsegmented. Of the eggs that had been in solution 3 (100 c.c. $\frac{1}{8}$ n $CaCl_2$) about five per cent were segmented into from 2 to 4 cells of very unequal size. The last examination had taken place in the evening. The next morning the eggs of solution 1 were teeming with blastulæ; some with regular, the majority, however, with most fantastic outlines (see Fig. 3, page 460). Their size was very unequal. I expected as much from the irregular appearance of the morule of the evening before. In the fertilized egg the membrane prevents any irregularity in the form of the blastulæ. The unfertilized eggs, however, have no membrane, and hence the cells are only kept together by an intercellular substance or by adhesion; but it is very probable that the processes of cell division are accompanied by amœboid motions (Fig. 2, page 459), which have the effect of making the arrangement of cells irregular. I have noticed and described this effect of the amœboid motions of the cleavage cells in my experiments on eggs whose membrane I had caused to burst and whose contents partly flowed out of the egg.¹ These extraovates behaved very much like the unfertilized eggs. In the latter case it was evident from the size of the blastulæ that only in rare cases had the whole egg developed into one single blastula. As a rule, each egg gave rise to several blastulæ. Through the amœboid motions connected with the process of cell division groups of cells became disconnected and developed into dwarf blastulæ. I shall discuss this point more fully in connection with the drawings.

I have not yet mentioned one control experiment which I made in this series. I had one part of the eggs of the same female fertilized and put into the same four solutions for the same time as the unfertilized eggs. Every one of the fertilized eggs formed a membrane. The behavior of these eggs and their larvæ differed also in other

¹ LOEB, J.: Archiv für Entwicklungsmechanik, 1895, i, p. 453.

respects from the larvæ produced from the unfertilized eggs. *While the blastulæ of the fertilized eggs even after the treatment with solution 1 swam at the surface of the sea-water, the parthenogenetic blastulæ were all at the bottom of the dish and unable to rise.* This difference seems to be typical, as I found it in all my experiments. All the parthenogenetic blastulæ in these experiments died during the day. It goes without saying that the blastulæ which developed from the fertilized eggs treated with solution 1 did not show the ragged condition of the parthenogenetic larvæ that had developed without a membrane.

Unfertilized eggs that had been in solution 2 for one hour and fifty minutes were the next day unsegmented and without membrane. The unfertilized eggs that had been in solution 3 were all dead. The unfertilized eggs that had been kept in normal sea-water all the time were without a membrane and unsegmented. Thus it is evident that the unfertilized eggs of *Arbacia*, if put for one hour and fifty minutes into a solution of 60 c.c. $\frac{2}{8} n$ $MgCl_2$ + 40 c.c. sea-water are able to develop into blastulæ which move about. But it is also evident from the control experiments that this cannot be due to the partial pressure or concentration of the Mg-ions alone, for in solution 2 (100 c.c. $\frac{1}{8} n$ $MgCl_2$) the concentration of the Mg-ions was almost the same as in solution 1, and yet no unfertilized egg was caused to segment by this solution. That the latter solution is not very poisonous for the *Arbacia* egg is shown by the fact that the fertilized eggs of *Arbacia* develop better in this solution than in 60 c.c. $\frac{2}{8} n$ $MgCl_2$ + 40 c.c. sea-water. Hence it is evident that the Mg-ions alone were not able to bring about the development of the unfertilized *Arbacia* eggs that had been in solution 1. Either the presence of other ions, such as are contained in the 40 c.c. of sea-water or the increased osmotic pressure in the mixture of 60 c.c. $\frac{2}{8} n$ $MgCl_2$ + 40 c.c. sea-water is essential for the development. The osmotic pressure of a $\frac{1}{8} n$ $MgCl_2$ solution is not very different from that of sea-water.

Second series. — It was, of course, my next task to repeat this experiment. I now knew which solution must be used in order to obtain parthenogenetic blastulæ, but I wanted to find out how long the eggs must remain in this solution in order to develop into a blastula. I put a quantity of unfertilized eggs of one female into a solution of 60 c.c. $\frac{2}{8} n$ $MgCl_2$ + 40 c.c. sea-water at ten o'clock. At various intervals a portion of these eggs was taken out of the solution and put back into 200 c.c. of normal sea-water. The first lot was put

back into normal sea-water after thirty minutes. No egg had a membrane and none was segmented. The second lot was put back into normal sea-water after sixty-five minutes, the third lot after one hour and forty minutes, the fourth after one hour and fifty minutes, the fifth after two hours and five minutes, the sixth after two hours and fifteen minutes, lot seven after two hours and thirty minutes, and the eighth lot after three hours and fifteen minutes. The first, second, and eighth lots differed much in regard to the time they were exposed to the artificial solution from lots 3, 4, 5, 6, and 7, which were taken out of the solution at much shorter intervals. No egg in any of these solutions had any membrane or showed any trace of segmentation at the time they were put back into normal sea-water. At 1.15 all the eggs were examined again. In the lots 3 to 7 many eggs were segmented into from 2 to 16 cells. The 16-cell stages were only found in lot 4. The rest had not gone beyond the 8-cell stage. In lot 2 very few eggs had segmented; in lot 1 all the eggs were segmented. *No egg had a membrane.* Another examination of the eggs was made at 3.05. In lot 1 about one per cent of the eggs was segmented in 2 cells; in lot 2 about five per cent of the eggs were divided, most of them into 2, some of them, however, into 8 or even 16 cells. In lot 3 about twenty per cent were segmented. Some had reached the 32-cell stage. In lot 4 more than twenty per cent were segmented, some as far as into 32 cells. In lot 5 almost every second egg was segmented. Some had reached about the 32-cell stage. The same was true of lots 6 and 7. In lot 8 many eggs had segmented, but they were far behind in their development. *No egg had a membrane.* The single cells did not stick as closely together as they did in the fertilized egg with the membrane. I was afraid, from the appearance of the eggs, that they would not give rise to blastulæ, inasmuch as it seemed as though the cleavage cells would all fall apart.

The eggs were not examined until the next morning. In lots 1 and 8 there were practically no blastulæ in motion, or if there were any they escaped my observation. In lot 7 I found a small number of blastulæ. In lots 3, 4, 5, and 6 the water at the bottom of the dish was teeming with blastulæ, which with their irregular outlines and the variation in size betrayed clearly that they had developed from eggs without a membrane.

In the afternoon I found living larvæ only in lots 3, 4, 5, and 6. Some larvæ seemed to be in a gastrula stage, and some even in the

transitional stage to the pluteus form. Many had died, and this accounted perhaps for the fact that no blastulæ were left in lots 2 and 7, where they had at most been very scarce. The next morning only a few larvæ were left in lots 3 to 6, and these died during the day.

I stated at the beginning of my experiments that only a part of the eggs of one female were put into the MgCl_2 solution. The others were left in normal sea-water to serve as control material. None of these eggs which were in the same sea-water as that used for the eggs treated with the MgCl_2 solution formed any membrane. After twenty-four hours a few eggs were found divided into 2 cells. No egg developed beyond this stage.

This experiment shows that the time during which unfertilized eggs must remain in contact with a mixture of 60 c.c. $\frac{2}{8} n$ MgCl_2 + 40 c.c. sea-water in order to give rise to blastulæ is limited in two directions. If the eggs remain only thirty minutes in such a solution, a few of them may begin to develop, but none will reach the blastula stage. But if the unfertilized eggs remain in this solution from one and a half to two hours, more than twenty per cent and as many as fifty per cent may develop, and the solution then teems with moving blastulæ, which however remain at the bottom of the dish. On the other hand the time limit will be exceeded if the eggs are left in the solution more than two and a half or three hours.

I tried another experiment in this series to see how soon the unfertilized eggs would lose their power of being affected by the MgCl_2 solution. The eggs were left in normal sea-water for eighty minutes, then put in a solution of 60 c.c. $\frac{2}{8} n$ MgCl_2 + 40 c.c. sea-water for two hours. Two hours later they were put back into normal sea-water. A large number of eggs began to segment, but I did not find any blastulæ the next day.

Third series. — Thus far I had found the right solution for producing blastulæ from unfertilized eggs, and had found about how long the eggs must remain in this solution. I now desired to verify these results and in addition find out accurately how far the proportions between sea-water and the $\frac{2}{8} n$ MgCl_2 solution might vary without interfering with the results. The unfertilized eggs of one female were distributed in the following solutions: —

1. 60 c.c. $\frac{2}{8} n$ MgCl_2 + 40 sea-water.
2. 30 " " + 70 "
3. Normal sea-water.

At five different periods (one hour, one hour and forty minutes, one hour and fifty-five minutes, two hours and twenty minutes, two hours and forty-five minutes) portions of the eggs in solutions 1 and 2 were brought back into normal sea-water. After all that has been said, it seems superfluous to give all the details as explicitly as in the preceding experiments, and I shall therefore confine myself to a description of the main results as they appeared next morning. The eggs in solution 3 (normal sea-water) had no membranes nor had any egg segmented. It is obvious that unfertilized eggs do not always undergo a beginning of segmentation in normal sea-water after twenty or twenty-four hours. Of the eggs that had been in solution 1 for one hour and fifty-five minutes, about twenty-five per cent had developed into a blastula which swam about. About the same result was obtained in the lot that had been for two hours and twenty minutes in solution 1. The appearance of these blastulæ was the same as in the previous experiments. Most of them were only fractions of one egg, and it was not uncommon to see four smaller blastulæ swim together, each apparently having developed from one of the blastomeres of the 4-cell stage. In the other lots which were taken from solution 1 a few blastulæ were formed. The eggs that had been in this solution for one hour were practically all undivided, except that one in a thousand had segmented into 2 to 3 cells. It was not much better in the lot of eggs that had been in this same solution for one hour and forty minutes. The lot that had been in the solution two hours and forty-five minutes had living blastulæ, but not so many as the two lots mentioned above. It is therefore obvious that the eggs of different females show slight variations in the time required for the eggs to remain in the mixture of 60 c.c. $\frac{2.0}{8} n \text{ MgCl}_2 + 40 \text{ c.c. sea-water}$ in order to reach the blastula stage.

I have thus far only spoken of eggs that had been in solution 1. Of the eggs that had been in solution 2 not one developed into a blastula. Those that had been in this solution for two hours had not even segmented. Only the eggs that had remained in that solution for two hours and forty-five minutes showed a beginning of segmentation (2 cells), but only one in a thousand had segmented. It is evident that either the amount of Mg-ions or the total osmotic pressure of the solution was too small to cause the unfertilized eggs to develop. These experiments with negative results are however very valuable as control experiments against the possible contamination of the sea-water with spermatozoa. If in such cases contami-

nation had happened, the eggs that had been in solution 2 ought to have developed equally well or better than those that had been in solution 1. The same remark might apply to the preceding and following experiments. *None of the eggs in any of these solutions formed a membrane.*

Fourth series.—In all the experiments in which blastulæ were produced from the unfertilized eggs three conditions were united: 1st, the total osmotic pressure of the artificial solution was higher than that of sea-water; 2nd, the amount of the Mg-ions was increased; 3rd, the absolute amount of the other ions normally present in sea-water was reduced. In this series I desired to find out whether the third condition was essential, and whether the mere increase in the osmotic pressure was not sufficient. Moreover I wished once more to repeat the former experiments. The unfertilized eggs of one female were distributed in the following solutions:—

1. 60 c.c. $\frac{2}{3}$ n $MgCl_2$ + 40 sea-water.
2. 100 c.c. sea-water + $3\frac{1}{2}$ gram (wet) $MgCl_2$.
3. 100 c.c. sea-water + 8 gram (wet) $MgCl_2$.
4. Normal sea-water.

At various intervals a lot of the eggs were taken out of each of the four solutions and put into normal sea-water. The eggs that had been in solution 1 from one and a half to two hours had developed into blastulæ the next morning. The number of blastulæ was comparatively larger than in any of my previous experiments. The eggs that had been in solutions 2 and 3 contained no blastulæ. Solution 2 is, by the way, the one Norman and Morgan had used in their experiments. In solution 4 no egg was even segmented the next day. In none of the four solutions had any egg formed a membrane. These experiments show that the substitution of a number of Mg-ions for one-half of the ions naturally contained in the sea-water is either necessary or more favorable than the mere addition of Mg-ions. This experiment explains why Morgan did not succeed in getting live larvæ, having treated the eggs with solution 2. But I intend to determine in my future experiments whether the addition of a little more than $3\frac{1}{2}$ grams of $MgCl_2$ and a little less than 8 grams of the solution to 100 c.c. of sea-water may not give more favorable results.

Fifth series.—I next wished to try whether it would not be possible to carry the artificial development of the unfertilized egg one step farther. The blastulæ thus far obtained were by no means healthy,

and although some of them looked normal they died before they had time to reach the pluteus stage. This latter result I was inclined to ascribe to the poisonous effect of the Mg-ions, and it seemed possible to me that a decrease in the amount of $MgCl_2$ and a slight increase in the amount of sea-water might allow the eggs to reach the pluteus stage. The eggs of two females were distributed in the following solutions:—

1. 60 c.c. $\frac{2}{3}$ N $MgCl_2$ + 40 sea-water.
2. 50 c.c. " + 50 "
3. Normal sea-water.

The eggs were brought back into normal sea-water after one hour and five minutes, one hour and thirty minutes, and one hour and forty minutes. Only the eggs of the last lot that had been in the solution one hour and forty minutes showed the beginning of a development. I believe that I took out the eggs too soon. In some cases such eggs are able to develop, but in others they are not, and I think it probable that if the eggs had been left a little longer in solution 1 or 2 they would have developed further. I made some camera drawings of the way in which the eggs were segmented (Fig. 2). The

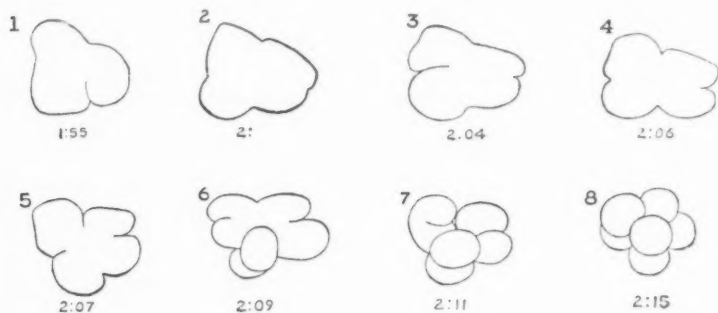


FIGURE 2. Successive amoeboid changes in an unfertilized egg that had been treated with a solution of 60 c.c. $\frac{2}{3}$ N $MgCl_2$ + 40 c.c. sea-water. The segmentation proceeded in 20 minutes from the unicellular stage into the 6-cell stage.

successive stages of the segmentation of one and the same egg up to the 6-cell stage were drawn. The reader will see from the drawings that the egg went within twenty minutes from practically an undivided egg into a 6-cell stage. It is obvious that these cell divisions are accompanied by most striking amoeboid motions, which are characteristic of all the eggs without a membrane. I believe that

these amœboid motions exist in the fertilized eggs just as well, but the membrane prevents them from becoming so conspicuous as in the unfertilized eggs where there is no membrane. In the normal eggs these amœboid motions are more symmetrical, and this is another reason why they escape our observation. When I made my first experiments on the effect of more concentrated sea-water upon

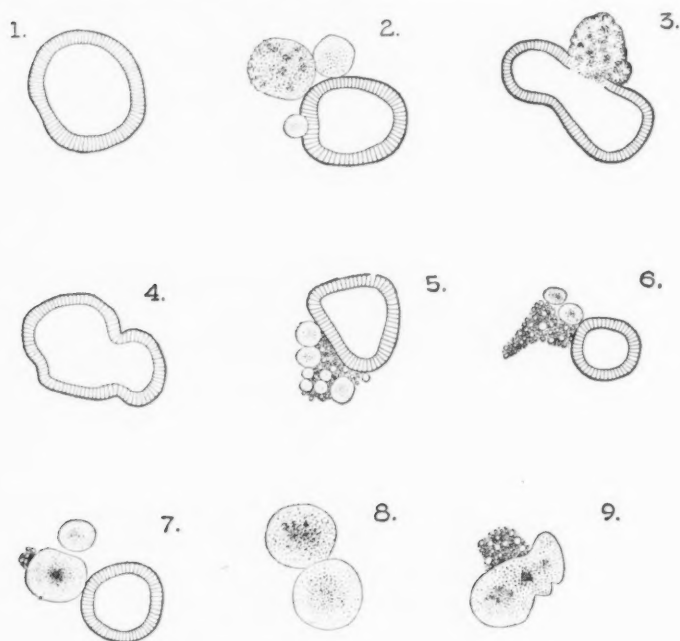


FIGURE 3. Camera drawings of various forms of parthenogenetic blastulæ.

Eggs treated with 60 c.c. $\frac{2}{3}N$ $MgCl_2$ + 40 c.c. sea-water. Each of these blastulæ had been in motion.

the segmentation of fertilized eggs, the idea struck me that the segmentation by budding (*Knospenfurchung*) was the outcome of amœboid motions, and I soon afterward expressed the idea that the same is true for the process of cell division in general.¹ The two nuclei of the mother cell are the centres around which the protoplasm streams and flows. These amœboid motions are only one episode in the pro-

¹ LOEB, J.: *Archiv für Entwicklungsmechanik*, 1895, i, p. 453.

cess of cell division, for whose full explanation other phenomena of an entirely different character must be taken into consideration.

Sixth series.—The preceding experiment was repeated, but this time with due consideration of the fact that the eggs must remain long enough (two hours) in the artificial solution. The eggs of two females were distributed in three solutions:—

1. 60 c.c. $\frac{2}{3}N$ $MgCl_2$ + 40 c.c. sea-water.
2. 50 c.c. " + 50 c.c. "
3. Normal sea-water.

None of the eggs formed a membrane. Some of those that had remained in normal sea-water segmented after twenty hours. They divided into from 2 to 3 cells and not further. I have already mentioned the fact that the unfertilized eggs of various females differ somewhat in their tendency to segment in normal sea-water. It may be possible that these variations enhance or diminish the effects of artificial solutions upon the development of unfertilized eggs. The eggs that had been for two hours in solution 1 had the next day developed into the characteristic blastulæ some of which are represented in Fig. 3. Some of these blastulæ originated possibly from the whole mass of one egg, for instance I, 3, and 4. But even here the irregular outline betrays clearly that the blastulæ originated from eggs without a membrane. As I said in an earlier experiment, the outlines of the eggs became irregular through the amoeboid motions of the blastomeres, and in the blastulæ the outline of the irregular morula stage is preserved. This is intelligible if we remember that the blastula originates through the

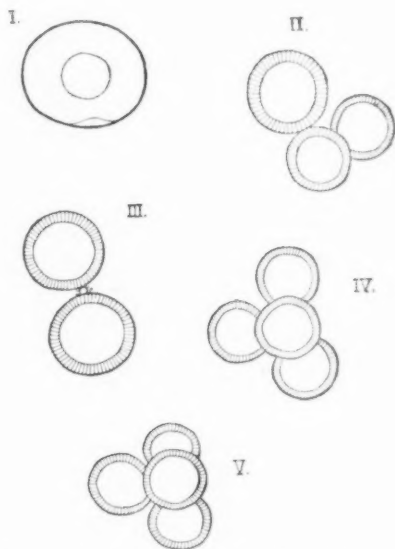


FIGURE 4. Camera drawings of parthenogenetic blastulæ. Eggs treated with equal parts of a $\frac{2}{3}N$ $MgCl_2$ solution and sea-water. Egg iv. and v. gave rise to quadruplets; ii, to triplets; iii, to twins.

cleavage cells moving or sticking to the periphery of the egg. The other blastulae represented only smaller pieces of a single egg. In some cases one part of the egg disintegrated and formed debris attached to the other part which reached the blastula stage (5, 6, and 7). Each one of these blastulae was moving and had to be immobilized to make the camera drawing. It is impossible to give a fair idea of the variety of forms of blastulae one sees in such experiments. No egg of this lot (solution 1) reached the pluteus stage. All died the second day. The eggs that had been in solution 2 (equal parts of $\frac{2}{8}$ n $MgCl_2$ and sea-water) looked very different from the preceding lot (Fig. 4). After twenty-four hours many of them had developed into blastulae. These blastulae left no doubt that they came from eggs without a membrane, inasmuch as in the majority of cases several blastulae originated from one egg. Quadruplets were especially frequent (Fig. 4, Nos. 4 and 5), but twins and triplets were also quite common. I watched their development, and am thus quite certain that these multiple embryos sticking together came from one egg. The feature that



FIGURE 5. Camera drawings of three parthenogenetic plutei. Eggs treated with equal parts of a $\frac{2}{8}$ n $MgCl_2$ solution and sea-water. The plutei are normal.

distinguished these embryos from those that had been treated with the stronger $MgCl_2$ solution, however, was the fact that the former all had regular and sharp outlines and were free from debris. The outlines of the blastulae were much more spherical. These blastulae had greater vitality than the others and kept alive during the next thirty-six hours. The next morning a number of them had reached the pluteus stage with a perfectly normal skeleton and intestine, but they died the following day (Fig. 5). They had lived more than two days. Their development was slower than in the case of fertilized eggs.

All these blastulæ and plutei swam about on the bottom of the dish, not rising to the surface like the larvæ from fertilized eggs.

The control eggs that had been left in the normal sea-water remained unsegmented, with the exception of a few which on the second day were found divided into 2 or 3 cells. The latter, of course, segmented no further. None of these eggs had a membrane.

Seventh series.—The preceding series had shown that a mixture of equal parts of $\frac{2}{8}^0$ *n* MgCl_2 and sea-water is more favorable for the development of the eggs than a mixture with more MgCl_2 and less sea-water, for instance 60 c.c. MgCl_2 and 40 c.c. sea-water. In the latter mixture the eggs seemed to suffer more. It must, however, be stated that as far as the comparative number of eggs is concerned that undergo development, the solution with 60 c.c. MgCl_2 and 40 c.c. sea-water is equally good or even better than the mixture of equal parts of both. I now tried whether a mixture with less MgCl_2 would still be favorable. *A mixture of 40 c.c. $\frac{2}{8}^0$ n MgCl_2 + 60 c.c. sea-water was found ineffective.* The eggs remained two hours in this solution, and a few of them segmented afterwards, but as the number was comparatively small I did not follow up this experiment. It is possible that a mixture of 40 c.c. $\frac{2}{8}^0$ *n* MgCl_2 + 60 c.c. sea-water is too weak to bring about artificial parthenogenesis of the egg of *Arbacia*. In one of the preceding experiments we found that by treating the eggs with a mixture of 30 c.c. $\frac{2}{8}^0$ *n* MgCl_2 + 60 c.c. sea-water we were not able to bring about parthenogenesis.

Eighth series.—It was evident that in order to produce plutei from the unfertilized egg of *Arbacia* we must confine ourselves to solutions which contain less than sixty and more than forty per cent of $\frac{2}{8}^0$ *n* MgCl_2 . In the next experiments the following four solutions were tried:—

1. 55 c.c. $\frac{2}{8}^0$ *n* MgCl_2 + 45 c.c. sea-water.
2. 50 " + 50 "
3. 45 " + 55 "
4. Normal sea-water.

At three different intervals (two hours, two hours and ten minutes, two hours and twenty minutes) portions of the eggs were taken out of these four solutions and put back into normal sea-water. Two hours later in each of the lots that had been in the first three solutions about fifty per cent of the eggs were segmented into from 2 to 16 cells. None of them had a membrane. No egg in solution 4

(normal sea-water) was segmented or had a membrane. The next morning the eggs that had been in solution 1 were teeming with blastulæ. Many of them resembled the blastulæ of Fig. 3, but the majority were clean and free from débris. The eggs that had been in solution 2 had a large number of blastulæ and gastrulæ. They were free from débris and looked very much like those drawn in Fig. 4. The eggs taken from solution 3 had very few blastulæ. The latter, however, were perfect, except that the single egg as a rule produced more than one embryo. The majority of the eggs were still in the morula stage. The next morning, forty-eight hours after the treatment with the $MgCl_2$ solution, each one of the three dishes contained perfect plutei. Many eggs of solution 3 which the previous day were still in the morula stage had in the mean time developed into blastulæ or plutei. This time the plutei were still alive on the following day (seventy-two hours after the treatment with the artificial solution). Their vitality was not much less than that of the normal plutei which often died just as early. I mentioned that I had put back the eggs from the $MgCl_2$ solutions into normal sea-water at three different intervals. Those taken out last gave the best results. It is very obvious that the unfertilized eggs develop much more slowly than the fertilized eggs. The latter reach the pluteus stage at the proper temperature within twenty-four hours or little more, while the unfertilized eggs reach the pluteus stage after forty-eight hours at the same temperature. I had the same experience in all my experiments with unfertilized eggs. The eggs that had been left in normal sea-water remained undeveloped and not one egg had a membrane. One egg in a hundred was segmented after twenty-four hours in 2 or 3 cells, but none developed further.

Ninth series.—This time I intended once more to repeat my experiments and at the same time make control experiments of an altogether different character. I will first speak of the repetition of the old experiments. The unfertilized eggs of one female were put into the following two solutions:—

1. 50 c.c. $\frac{2}{3}$ N $MgCl_2$ + 50 c.c. sea-water.
2. Normal sea-water.

Two hours later the eggs from solution 1 were put back into normal sea-water. Three and a half hours later about fifty per cent of the eggs that had been in solution 1 were divided into from 2 to 16 cells, but not an egg had a membrane. The control eggs that had

been in normal sea-water all the time were all without membrane and absolutely unsegmented. Millions of eggs were examined under the microscope. The next morning the eggs that had been in solution 1 had reached the blastula stage and were swimming about. A small number were in a gastrula stage and even beginning to assume a pyramidal form. In the control eggs not one had developed. Perhaps one in a hundred had amœboid forms such as precede segmentation in unfertilized eggs, but not an egg was segmented and not one had a membrane. The next day some of the blastulæ of the other lot had reached the pluteus stage. The control experiments will be discussed in the next paragraph.

Possible sources of error and objections.—From all these experiments I draw the conclusion that by putting the unfertilized eggs of *Arbacia* for two hours into a solution of 60 c.c. $\frac{2}{8} n$ $MgCl_2$ + 40 c.c. sea-water the eggs develop into blastulæ if brought back into normal sea-water. If we put the unfertilized eggs for about two hours into a solution of equal parts of $\frac{2}{8} n$ $MgCl_2$ and sea-water, the eggs may reach the pluteus stage. The possible objection might be that the eggs were fertilized. Such a fertilization could only have been caused by the instruments or hands of the experimenter having been in contact with spermatozoa or by the sea-water containing spermatozoa. The first possibility was absolutely excluded through the above mentioned precautions. The second possibility was rendered practically impossible, as, first, the spawning season was practically over, and, second, the spermatozoa lose their power of fertilizing eggs in a very short time (in about five hours). But that it was absolutely excluded is proven by the following facts:—

1. None of the unfertilized eggs kept in normal sea-water developed or formed a membrane. I examined millions of eggs in each experiment. Not one was found that was fertilized. The sea-water used in this case was the same as that used for the unfertilized eggs that did develop. If the sea-water had contained spermatozoa, the unfertilized eggs kept in normal sea-water all the time should have been fertilized.

2. None of the eggs which developed after treatment with $MgCl_2$ solution ever had a membrane. Fertilized eggs which were put immediately after fertilization into a mixture of equal parts of $\frac{2}{8} n$ $MgCl_2$ and sea-water and kept there for two hours did not lose their membrane. In the ninth series I made the following control experiments. Unfertilized eggs that had been in the above mentioned $\frac{2}{8} n$ $MgCl_2$

solution for two hours were put into normal sea-water to which fresh sperm was added. In this case a number of eggs formed membranes.

3. No blastula originated from an egg that had been kept for some time in one of the following solutions: —

1. 100 c.c. $\frac{1}{8}$ *n* MgCl_2 .
2. 30 c.c. $\frac{2}{3}$ *n* " + 70 sea-water.
3. 40 " " + 60 "
4. 100 c.c. sea-water + $3\frac{1}{2}$ grams (wet) MgCl_2

and the solutions mentioned in Chapter III. Yet eggs of the same female that had been kept for some time in a mixture of 50 or 60 c.c. $\frac{2}{3}$ *n* MgCl_2 and 50 or 40 c.c. of sea-water developed into blastulæ or plutei. This happened in spite of the fact that the vitality of the latter eggs had suffered more than that of those in the above mentioned solutions with more sea-water and less MgCl_2 . Moreover the water was always changed in both classes of eggs simultaneously, and the chances for fertilization of the eggs from spermatozoa contained in the sea-water were equal for both. If the sea-water had contained any spermatozoa capable of impregnating the eggs, those eggs that had been in solutions with less MgCl_2 should have been fertilized first.

4. In almost all the experiments eggs were taken out of the mixture of 60 to 50 c.c. $\frac{2}{3}$ *n* MgCl_2 + 40 to 50 c.c. sea-water at different periods. In no case did a single egg develop into a blastula that had been in this solution for less than one half hour, and generally only those eggs yielded blastulæ that had been in this solution for about two hours. If the sea-water had contained spermatozoa, the latter should have fertilized those eggs first which had been a shorter time in the artificial solution. On the other hand the eggs that had been left in the artificial solution more than two and a half hours as a rule yielded fewer or no blastulæ.

5. I stated above that even at the height of the spawning season eggs are rarely fertilized by spermatozoa contained in the running sea-water. I do not think one would be likely to see more than one egg in a thousand undergo development under such conditions, provided that no contamination through the instruments occurred. In our experiments which were made at the end of the spawning season about twenty to fifty per cent of the eggs that had been kept in the right solution developed. It is out of the question to attribute such a result to spermatozoa contained in the sea-water.

6. As far as I can see, there is only one possible source of error left. It might be that the sea-water contained spermatozoa, but that these spermatozoa were not able to fertilize *normal eggs*, while a treatment of the egg with the mixture of 60 c.c. $\frac{2}{8} n$ $MgCl_2$ + 40 c.c. sea-water increased its impregnability, or a treatment of the spermatozoon with the same solution increased the fertilizing power of the spermatozoon. Both possibilities must, however, be discarded. As far as the impregnability of the egg is concerned, I made the following experiments in the last series. Unfertilized eggs were put into a solution of 50 c.c. $\frac{2}{8} n$ $MgCl_2$ + 50 c.c. sea-water and left in this solution for two hours. They were then taken out and fertilized with fresh spermatozoa. At the same time another lot of the eggs of the same female which had been kept for two hours in normal sea-water were fertilized with sperm of the same male. Practically every egg of the latter lot developed into a blastula, while only about fifty per cent of those eggs that had been in the $MgCl_2$ solution reached the blastula stage. *Hence the treatment with $MgCl_2$ diminishes the power of development of eggs, but does not increase it.* As far as the spermatozoa are concerned, former experiments by Norman, Morgan, and myself showed that a slight increase in the concentration of the sea-water abolishes the fertilizing power of spermatozoa very rapidly. In my experiments I added 2 grams of NaCl to 100 c.c. of sea-water. "The spermatozoa which had been in this solution for only a few hours, when brought back into normal sea-water, fertilized only a thousandth part or less of the normal eggs, while the spermatozoa of the same animal which had remained in normal sea-water fertilized at the same time almost all the eggs."¹ Morgan repeated my experiments, obtaining the same result.² Norman tried the effects of a slight increase of $MgCl_2$ upon spermatozoa.³ I repeat his statement: "I put sperm at 8.30 into $MgCl_2$ solution $2\frac{1}{2}$ grammes to 100 c.c. of sea-water. At 8.30 some of the sperm was mixed with normal unfertilized eggs, and within three minutes the eggs were fertilized. At 8.42 eggs and sperm were again mixed. In two minutes egg membranes began to become visible, showing normal fertilization, and within another minute all the eggs were fertilized. At 8.52 another test was made, but at this time the egg membrane did not appear, showing that fertilization did not take place. At 9.00 o'clock about one egg

¹ LOEB, J.: Journal of morphology, 1892, vii, p. 253.

² MORGAN, T. H.: Anatomischer Anzeiger, 1894, ix, p. 141.

³ NORMAN, W. E.: Archiv für Entwicklungsmechanik, 1896, iii, p. 106.

in every 100 was fertilized." Norman repeated these experiments several times with the same result. They prove that even a small addition of MgCl_2 to sea-water, much smaller than in any of our experiments, suffices to annihilate the power of impregnation in the spermatozoa in a very short time. In my own experiments the increase in the osmotic pressure of the sea-water was much greater than in Norman's experiments. I made another control experiment in the ninth series which bears on the same question. Unfertilized eggs were left in a solution of equal parts of $\frac{2}{3} n$ MgCl_2 and sea-water for two hours. At the end of that time they were put back into normal sea-water to which sperm was added which had also been in a solution of equal parts of $\frac{2}{3} n$ MgCl_2 and sea-water for two hours. Only very few of the eggs formed a membrane.

There is, as we saw, a typical difference between the blastulæ and plutei which develop from fertilized and unfertilized eggs. The former rise to the surface, the latter swim at the bottom of the dish. If eggs be kept for two hours in the MgCl_2 solution and then fertilized with normal sperm, the blastulæ rise to the surface. If they be fertilized with sperm that had been in MgCl_2 solution for two hours, they remain at the bottom of the dish like the unfertilized eggs. It is thus clear, I think, that even this last possible objection that the treatment with the MgCl_2 solution increases the impregnating power of the spermatozoa or the impregnability of the egg must be discarded. Hence I draw the conclusion that the unfertilized eggs that had been treated with equal parts of $\frac{2}{3} n$ MgCl_2 and sea-water developed parthenogenetically.

V. SOME REMARKS CONCERNING THE NATURE OF THE PROCESS OF FERTILIZATION.

The facts of the preceding chapter force us to transfer the problem of fertilization from the realm of morphology into that of physical chemistry. There is certainly no reason left for defining the process of fertilization as a morphological process. The morphology of the spermatozoon itself becomes of secondary importance as far as the process of fertilization is concerned.

The spermatozoon not only starts the development of non-parthenogenetic eggs, but it is also the bearer of the hereditary qualities of the male. From our experiments it becomes evident that these two functions of the spermatozoon are not necessarily bound together, for nobody would assume for an instant that the hereditary qualities that

are carried by the spermatozoon could be imparted to the egg by a change in the inorganic constituents of the sea-water. We have learned to attribute the different activities of a cell to different enzymes. We must in future consider the possible or probable separation of the fertilizing qualities of the spermatozoon from the transmission of hereditary qualities through the same.¹

The plutei produced from the unfertilized egg resemble closely in every regard those produced from the fertilized egg. The latter in many cases live longer than the former, but even this was not so in every case, and it is not impossible that in further experiments parthenogenetic plutei with a greater duration of life will be produced. The only difference between parthenogenetic and normal blastulae is that the latter rise to the surface of the water, while the former do not. One might think that this was due to the influence of the $MgCl_2$ solution on the egg. This is, however, not the case. Eggs that had been in such a solution and were fertilized afterwards rose to the surface. Even this difference might be caused to disappear by further experimentation.

An agency which causes the egg to go through only the first stages of segmentation, which lead, for instance, to a division of the egg into 2, 4, or 8 cells, need not necessarily have much in common with those agencies in the spermatozoon that cause the development of the fertilized egg. But if the egg can be caused through an artificial influence to reach the blastula stage and swim about, the artificial cause must have more in common with the effective element in the spermatozoon. If however the artificial influences cause the egg to reach the pluteus stage, or in other words cause the egg to develop as far as the fertilized egg can be developed at present in our laboratory, I think the two processes of artificial and natural development must be pretty closely allied.

It is in harmony with our statement that a very large number of conditions cause an unfertilized egg to reach a 2- or 4- cell stage. It suffices to leave the eggs for some time in sea-water (about twenty-four hours). A slight increase in the alkalinity of the sea-water causes the beginning of a segmentation much sooner. A short treatment with sea-water that is neutral or faintly acid has the same effect. An increase in the concentration of the sea-water which probably causes a loss of water in the egg has the same effect (Morgan). Morgan

¹ LOEB: The heredity of the marking in fish embryos. Woods Hole Biological Lectures, Boston, 1899.

found more recently that treatment with a solution of strychnia salts may lead to a beginning of segmentation.¹ Possibly in this case the alkalinity of the sea-water was modified. But none of these or the other methods mentioned above has yielded blastulæ, gastrulæ, or plutei.

There is at present only one way known by which the unfertilized egg of *Arbacia* can be caused to develop into a pluteus.² This consists in treating the unfertilized eggs for two hours with a mixture of about equal parts of a $\frac{2}{8} n$ $MgCl_2$ solution and sea-water. It is of theoretical interest to find how this treatment may possibly affect the egg substance. The bulk of our protoplasm consists of proteids, which according to their physical behavior belong to the colloidal substances. The proteids are characterized by two qualities which are of the utmost importance in the analysis of life phenomena. The proteids change their state very easily, and readily take up or lose water. It is more than probable that one or both of these qualities may account for muscular contractility and protoplasmic motion. The agencies which affect these two variable qualities of the protoplasm most powerfully are, first of all, certain enzymes (for instance, plasmare, trypsin, etc.). Almost equally powerful are ions in certain concentrations. As I have dwelt upon this point in my three preceding publications,³ it need not be repeated here. But I wish to call attention to a most interesting paper by Dr. E. Pauli, which has recently appeared and which throws more light on this subject.⁴ The third agency is the temperature.

In our experiments it was evidently the second factor which affected the condition of the colloids. The transitory treatment of the unfertilized eggs with a mixture of equal parts of a $\frac{2}{8} n$ $MgCl_2$ solution and sea-water brings about a change in the physical conditions of certain colloids which is not reversed by putting them back into normal sea-water, and which allows them to develop into normal plutei.

As far as the spermatozoon is concerned, it may bring about the same change in the condition of the colloids in the egg, either by its carrying specific ions into the egg or by carrying enzymes, or in

¹ MORGAN, T. H.: *Science*, 1900, N. S., xi, p. 176.

² I have not been able to raise the *fertilized* eggs of *Arbacia* beyond the pluteus stage in the laboratory.

³ LOEB: This journal, 1900, iii, pp. 135, 327, and 383.

⁴ PAULI: *Archiv f. d. ges. Physiol.*; 1899, lxxviii. p. 315.

some other way which is as yet unknown to us. It is certainly remarkable that the spermatozoa contain a large amount of ash (five per cent, according to Hammarsten). In the parthenogenetic egg the colloids are from the beginning in such a condition as to allow the development to proceed. In other animals it is perhaps solely the ion constitution of the sea-water or of the blood which prevents the eggs from developing parthenogenetically. I shall discuss this point more fully in connection with further experiments on this subject.

ON THE MAXIMUM PRODUCTION OF HIPPURIC ACID IN RABBITS.

BY W. H. PARKER AND GRAHAM LUSK.

[From the Chemical Laboratory of the Yale Medical School.]

IT has long been known that glycocoll is a decomposition product of certain protein substances and that gelatine yields this body among its decomposition products. Fischer¹ has furnished a quantitative method for the determination of glycocoll and Gonnermann² has shown that about nine per cent of glycocoll can be split off from gelatine. Collagen and spongin also yield glycocoll and Spiro³ has recently obtained it as a decomposition product of the hetero-albumose from fibrin.

Glycocoll combines with benzoic acid within the organism to form hippuric acid, which is excreted from the body in the urine. It is also well known that hippuric acid occurs in large amounts in the urine of herbivora but only in small quantities in that of carnivora. The large quantity of hippuric acid present in the urine of herbivora may be partly explained by the fact that vegetable foods contain large amounts of aromatic substances. Toluol, cinnamic acid and hydrocinnamic acid by oxidation, and also quinic acid by reduction, may be converted into benzoic acid within the organism. The benzoic acid so formed in the organism increases the output of glycocoll by increasing the ability to form hippuric acid. A small amount of hippuric acid is excreted in human urine on a mixed diet. The quantity is increased after a diet of vegetables and fruits probably in exactly the same manner as suggested above in the case of herbivora. Salkowski⁴ even found hippuric acid in the urine of starving dogs, as well as in the urine of dogs fed exclusively upon a diet of meat. This is explained by the fact that phenylacetic and phenylpropionic acids are formed in small quantities, in proteid putrefaction, and these are subsequently burned to form benzoic acid which combines with glycocoll.

¹ FISCHER: *Zeitschrift für physiologische Chemie*, 1894, xix, p. 164.

² GONNERMANN: *Archiv f. d. ges. Physiol.*, 1895, lix, p. 42.

³ SPIRO: *Zeitschrift für physiologische Chemie*, 1899, xxviii, p. 174.

⁴ SALKOWSKI: *Berichte der deutschen chemischen Gesellschaft*, 1878, xi, p. 500.

The several facts enumerated above show that the amount of hippuric acid depends upon the amounts of benzoic acid formed or present in the organism. The excess of glycocholl, over and above the amount necessary to unite with the benzoic acid produced from the diet, may be transformed into urea within the organism; as Schultzen and Nencki¹, and Salkowski² have demonstrated.

It was with the primary view of determining whether glycocholl was a cleavage product of proteid, and if so in what quantity it was produced, that the present investigation was undertaken. It has been shown by Reilly, Nolan, and Lusk³ that about sixty per cent of the proteid molecule is resolvable into dextrose. This would leave a nitrogen-containing remainder of forty per cent in which the carbon atoms are in relation to nitrogen, as follows; C : N :: 2.2 : 1; and it may be theoretically possible that this nitrogenous remainder of the proteid contains, in part at least, the radical of glycocholl, a substance in which the atoms C and N bear the quantitative relation 2 : 1.

In our experiments benzoic acid (in the form of lithium benzoate) was administered in excess to rabbits in order to form hippuric acid with the glycocholl believed to be split off in proteid metabolism. It is known that glycocholl is a product of general metabolism. In dog's bile there is no glycocholic acid, whereas feeding benzoic acid brings about an immediate excretion of hippuric acid in the urine. Moreover, Bunge and Schmiedeberg⁴ long ago pointed out that benzoic acid and glycocholl were synthesized to hippuric acid in the kidney of the dog but in no other part of the animal. In the rabbit, however, Salomon⁵ has found that the formation of hippuric acid seems to take place in other organs such as liver and muscles. The synthesis of hippuric acid is therefore not exclusively limited to any special organ, in rabbits.

Van der Velde and Stokvis⁶ have shown that in the rabbit no secondary decomposition of hippuric acid into the original glycocholl and benzoic acid takes place, as has been observed to occur in dogs.

¹ SCHULTZEN and NENCKI: *Zeitschrift für Biologie*, 1872, viii, p. 124.

² SALKOWSKI: *Zeitschrift für physiologische Chemie*, 1880, iv, pp. 55 and 101.

³ REILLY, NOLAN, and LUSK: *This journal*, 1898, i, p. 395.

⁴ BUNGE and SCHMIEDEBERG: *Archiv für exper. Pathol. u. Pharmacol.*, 1877, vi, p. 233.

⁵ SALOMON: *Zeitschrift für physiol. Chemie*, 1879, iii, p. 365.

⁶ VAN DER VELDE and STOKVIS: *Archiv für exper. Pathol. und Pharmacol.*, 1883, xvii, p. 189.

In the light of this knowledge Wiener¹ has made some experiments with fasting rabbits. He found in general that a dose of benzoic acid of 1.7 grams per kilogram of body weight produced fatal results but that doses of 1.0 to 1.5 grams may be given with impunity, and that after administering per os one gram of benzoic acid a maximum of hippuric acid is excreted; an amount which cannot be increased by giving any additional benzoic acid. The quantity of hippuric acid is very constant; for one gram of benzoic acid ingested 0.78 to 0.83 gram of hippuric acid per kilogram of body weight is excreted. This indicates that in rabbits the glycocoll supply of the organism is very small. When small doses of benzoic acid were ingested all the benzoic acid re-appeared in the urine combined with glycocoll. Wiener collected the urine for four days following a dosage of benzoic acid in excess and found quite a constant excretion of between 0.3276 and 0.3496 gram of glycocoll per kilogram of body weight. He regards this as the body's reserve glycocoll.

Wiener also observed that subcutaneous injection of glycocoll increases the amount of hippuric acid in the urine; while ammonium lactate, butyrate, diamido-propionic acid, and carnitic acid have no influence. He concludes that glycocoll normally exists in small amounts in the body but that it is not an intermediate product of proteid metabolism.

In the experiments cited below, benzoic acid was not given in a single dose as in Wiener's experiments, but frequently administered in small doses so that benzoic acid could always be absorbed from the alimentary canal, and thus allow a synthesis with any glycocoll which might be formed. The hippuric acid nitrogen was further compared with the total nitrogen eliminated, so as to indicate the relation between the glycocoll split off and the proteid metabolism of the body.

The experiments were done at first upon fasting animals, and subsequently the effect of various foods was noted. The urine was collected through a catheter and the bladder was carefully washed with warm water after the final collection of urine for a particular day.

Before discussing the results of the different experiments a convenient modification of the method of Bunge and Schmiedeberg² will be described which was found after many trials to be more expeditious and more accurate than theirs.

¹ WIENER: *Archiv für exper. Pathol. und Pharmakol.*, 1898, xl, p. 313.

² BUNGE and SCHMIEDEBERG: *Ibid.*, 1877, vi, p. 233.

DETERMINATION OF HIPPURIC ACID IN URINE.

One hundred cubic centimetres of the urine are evaporated nearly to dryness with 5 c.c. of saturated solution of sodium carbonate and 2 grams fine animal charcoal. The residue is taken up with boiling water, thoroughly rubbed with a rubber-tipped glass rod, and filtered through animal charcoal into a separating funnel. The charcoal residue and filter paper are carefully washed with hot water but the total filtrate should not exceed 50-60 c.c. The filtrate is next acidified with concentrated hydrochloric acid and allowed to cool, after thoroughly shaking to drive off the carbon dioxide. The hippuric acid, if present in considerable quantity, crystallizes out on cooling.

The hippuric acid is then extracted with acetic ether. The first extraction is made with 50 c.c. unwashed acetic ether and is followed by three extractions with 25 c.c. each of washed acetic ether. Care must be taken not to shake too hard in extracting, as a gelatinous mixture may result which will not separate even on long standing.

Each extract is drawn off into a separating funnel containing 25 c.c. of water, which has been previously saturated with acetic ether. The united washed extracts are evaporated nearly to dryness over the water bath and then allowed to stand at room-temperature, when the acetic ether volatilizes and the hippuric acid crystallizes out.

The dried hippuric acid is next separated from any benzoic acid by adding 25 c.c. chloroform and 5 c.c. benzol. It is then transferred to a dried and weighed filter paper, washed with chloroform and benzol mixture, and dried to constant weight in a weighing bottle.

The method was tested in the following series of experiments: —

TABLE I.
Mixed Sterilized Human Urine.

Urine taken Cubic Centimetres.	Hippuric Acid added in Grams.	Hippuric Acid Recovered.		
			After deducting 0.0009 Grams.	Per cent.
100	0	.0602		
100	0	.0616		
100	.200	.2552	.1943	97.1
100	.300	.3515	.2906	96.9
100	1.00	.9715	.9106	91.1
100	1.00	.9941	.9332	93.3

EXPERIMENTS ON FASTING RABBITS.

Turning now to the actual experiments, a preliminary investigation is presented below which shows the relation between hippuric acid nitrogen and total nitrogen in an ordinary fasting rabbit. Since phenylacetic and phenylpropionic acid may occur in the intestinal tract as products of the putrefaction of mucin even during fasting there must normally be traces of hippuric acid in the urine (Table II).

TABLE II.

Rabbit = 2.17 Kilo.

	Urine in Twenty-four Hours.	Total Nitrogen in Grams.	Hippuric Acid extracted in Grams.	Ratio of Nitrogen of Hippuric Acid to Total Nitrogen.
3d day of fast.	45 c.c.	1.384	.131	1 : 13.8
4th " "	80 "	1.169	.047	1 : 31.6
5th " "	65 "	.403	.038	1 : 13.9
6th " "	70 "	.696	.041	1 : 23.2

The following third and fourth tables indicate the effect of the administration of benzoic acid twice a day during fasting, in amounts sufficient to eliminate the glycocoll in combination as hippuric acid but not sufficient to produce toxic symptoms. The ratio obtained, after the first dose of benzoic acid, is much lower throughout all the experiments than the succeeding ratios and may be explained as a sweeping out of an actual surplus of glycocoll present at the time of administration of benzoic acid. In fact, in all the experiments the precaution was taken first to sweep out the excess of glycocoll before beginning any particular diet.

In Table III the results are quite irregular. Leaving out of the calculation those fasting days where the extremely high and low ratios of hippuric acid nitrogen to total nitrogen run from 1 : 64 to 1 : 9.2 we have three days in which the ratios run with the following numbers: 14.2, 18.4, 15.8, an average of 1 : 16.1. In Table IV, using another rabbit, the results are more uniform. Leaving out the first

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TABLE III.
Rabbit = 1.7 Kilos.

	Urine in Twenty- four Hours.	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Hippuric Acid excreted in Grams.	Ratio of Nitrogen of Hippuric Acid to Total Nitrogen.
3d day of fast.	47 c.c.	0.960			
4th " "	106 "	.947	.5 gm. 9 A. M. .5 " 9 P. M.	1.0844	1 : 11.1
5th " "	100 "	.984	.5 " 9 A. M. .2 " 9 P. M.	.8800	1 : 14.2
6th " "	68 "	.649	.4 " 9 A. M.	.1304	1 : 64.8
7th " "	90 "	.570	.7 " 9 A. M.	.3985	1 : 18.4
8th " "	42 "	.294	.4 " 9 A. M.	.3968	1 : 9.2
9th " "	52 "	.346	.4 " 9 A. M.	.2816	1 : 15.8

The animal exhibited toxic symptoms on the tenth day immediately after the ingestion of the benzoic acid, and died at 11 a. m., although 0.4 gram of glycocoll was given subcutaneously.

TABLE IV.
Rabbit = 1.5 kilo.

	Urine in Twenty- four Hours.	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Hippuric Acid excreted in Grams.	Ratio of Nitro- gen of Hippuric Acid to Total Nitrogen.
3d day of fast.	34 c.c.	0.833		0.040	1 : 26.6
4th " "	99 "	1.065	.5 gm. 9 A. M. .5 " 9 P. M.	0.9664	1 : 14.0
5th " "	70 "	0.990	.5 " 9 A. M.	0.7060	1 : 18.0
6th " "	107 "	1.087	.5 " 9 A. M.	0.6340	1 : 21.6
7th " "	100 "	0.775	.4 " 9 A. M.	0.4944	1 : 19.8
8th " "	108 "	1.148	.2 " 9 A. M. .2 " 9 P. M.	0.5760	1 : 25.5
9th " "	45 "	0.515	.4 " 9 A. M.	0.3252	1 : 21.8

The animal exhibited toxic symptoms on the tenth day and died during the day.

day when the ratio is always low on account of the sweeping out of the body's store of glycocoll, the ratio numbers run: 18.0, 21.6, 19.8, 25.5, 21.8, or an average of 1:21.5. This second experiment indicates that an average of 4.65 per cent of the total nitrogen in the metabolism of starvation may appear in glycocoll, or that 3.98 grams of glycocoll may arise from the decomposition of 100 grams of body proteid.

The total quantity of glycocoll per kilogram of body weight eliminated in four days greatly exceeds that which Wiener¹ found during his collection of urine for the same period, being 1.77 and 1.75 grams instead of 0.3276 and 0.3496 gram. Wiener, in his experiment, gave a single large dose of benzoic acid and did not follow this up with small doses.

Horses' urine taken at random has shown the following ratios:—

Hippuric Acid Nitrogen : Total Nitrogen = 1 : 15
Hippuric " " " = 1 : 17

INFLUENCE OF CARBOHYDRATES.

Since it occurred to us that there might possibly be a production of acetic acid in the metabolism of carbohydrates, followed by its combination with ammonium salts to form glycocoll synthetically, experiments with carbohydrate feeding were devised. In a first experiment rice was fed alone, and the quantity of hippuric acid was not found to vary from the normal in fasting, as Table V shows.

TABLE V.
Rabbit = 2.2 Kilos.

	Urine in Twenty- four Hours.	Rice Fed in Grams.	Total Nitrogen in Grams.	Hippuric Acid excreted in Grams.	Ratio of Nitro- gen of Hippuric Acid to Total Nitrogen.
3d day of fast.	45 c.c.		1.38	0.0102	1 : 138
1st day rice feeding.	80 "	50	1.17	.0037	1 : 316
2d day rice feeding.	65 "	50	.40	.0029	1 : 139
3d day rice feeding.	70 "	50	.70	.0031	1 : 232

¹ WIENER: *loc. cit.*

Into another animal benzoic acid was ingested and rice given, but the animal refused to eat the rice. After repeated trials the rice diet was abandoned and an experiment was carried on with carrot feeding and constant dosage with benzoic acid.

TABLE VI.

	Carrot fed in Grams.	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Hippuric Acid excreted in Grams.	Ratio of Nitrogen of Hippuric Acid to Total Nitrogen.
3d day of fast.		1.07		0.070	1 : 195
4th " "		1.20	.50 gm. 9 A. M. .50 " 9 P. M.	1.202	1 : 12.7
5th " "		1.17	.50 " 9 A. M. .25 " 9 P. M.	.991	1 : 15.1
1st day feeding	150.	1.50	.25 " 9 A. M.	.315	1 : 60.6
2d " "	80.	1.24	.50 " 9 A. M. .50 " 9 P. M.	.607	1 : 26.0
3d " "	40.	1.52	.25 " 9 A. M. .25 " 9 P. M.	.462	1 : 41.9
4th " "	25.	1.94	.50 " 9 A. M.	.107	1 : 23.0
5th " "	50.	2.15	*	.082	1 : 33.6

* Animal too weak to take any more benzoic acid but later ate the carrot.

The ratio here between total nitrogen and glycocholic nitrogen is quite variable, but it does not in any case point to an increase in the amount of glycocholic formed after feeding carbohydrates. It must therefore be that carbohydrates are not converted into glycocholic.

INFLUENCE OF GELATINE AND CASEIN.

It has already been stated that glycocholic may be derived from gelatine by laboratory methods even to the extent of 8.44 per cent. In other words about ten per cent of the total nitrogen of gelatine can be split off as glycocholic. It might be possible that the glycocholic of urinary hippuric acid is largely derived from gelatine, and might largely increase after feeding gelatine to a rabbit under the influence of benzoic acid. In fact the elimination of glycocholic might perhaps be a measure for gelatine decomposition in the body. The following table indicates the results obtained after feeding gelatine and benzoic acid.

The gelatine was dissolved in warm water and introduced into the stomach of the rabbit through a sound.

TABLE VII.
Rabbit = 2.2 Kilos.

	Gelatine fed in Grams.	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Hippuric Acid excreted in Grams.	Ratio of Nitrogen of Hippuric Acid to Total Nitrogen.
2d day of fast.		1.08	.50 gm. 9 A. M. .50 " 9 P. M.	1.014	1 : 13.7
3d " "		1.37	.50 " 9 A. M. .25 " 9 P. M.	0.741	1 : 23.6
1st day feeding.	4	1.75	.25 " 9 A. M. .50 " 2 P. M. .50 " 9 P. M.	.863	1 : 25.8
2d " "	5	2.42	.25 " 9 A. M. .50 " 2 P. M. .50 " 9 P. M.	1.109	1 : 27.9
3d " "	10	2.28	.50 " 9 A. M. .50 " 9 P. M.	.965	1 : 30.1
1st day of fast.			.25 " 9 A. M.		

The animal immediately after the ingestion of the benzoic acid showed toxic symptoms and died at 2 P. M.

On the days of gelatine feeding the considerable rise of nitrogen in the urine indicates the absorption and metabolism of gelatine. During these days the ratios between the glycocholic nitrogen and the total nitrogen were 1 : 25.8, 1 : 27.9, and 1 : 30.1, an average of 1 : 27.9. This indicates that after feeding gelatine about 3.58 per cent of the nitrogen belonging to proteid (and gelatine) metabolized in the tissues is eliminated as glycocholic, or 3.08 grams of glycocholic from the mixed decomposition of proteid and gelatine. Since 3.98 grams of glycocholic may arise from proteid in the fasting animal, it is seen that gelatine feeding certainly does not increase the glycocholic excretion over what is produced from the body's proteid.

The next experiment was with casein to see whether this ingested proteid would give the same ratio as the body's proteid. The casein was dissolved in warm water and introduced into the stomach through a sound.

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TABLE VIII.
Rabbit = 1.9 Kilos.

	Casein fed in Grams.	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Hippuric Acid excreted in Grams.	Ratio of Nitro- gen of Hippuric Acid to Total Nitrogen.
2d day of fast.		0.832		0.0580	1 : 18.5
3d " "		1.332	.5 gm. 9 A. M. .5 " 9 P. M.	.9115	1 : 18.5
1st day feeding.	4	1.469	.5 " 9 A. M. .5 " 9 P. M.	.7230	1 : 25.9
2d " "	5	1.456	.5 " 9 A. M. .5 " 9 P. M.	.7575	1 : 24.6
3d " "	10	1.929	1.0 " 9 A. M. .5 " 9 P. M.	1.0302	1 : 23.9

After feeding casein to the rabbit with simultaneous benzoic acid feeding, the ratios between hippuric acid nitrogen and total nitrogen were found to be 1 : 25.9, 1 : 24.6, and 1 : 23.9, an average of 1 : 24.8. This indicates that, after casein feeding, 4.03 per cent of the total nitrogen in the proteid metabolized may occur as glycocholl, or 3.45 grams of glycocholl for every 100 grams of proteid destroyed. This result does not greatly vary from the 3.98 grams of glycocholl obtained from every 100 grams of the body's proteid.

PHLORHIZIN EXPERIMENTS.

It was thought that the combined administration of phlorhizin and benzoic acid would show an increase of hippuric acid in the fasting rabbit proportioned to the increase of the proteid metabolism in diabetes. The result of the first experiment is shown in Table IX.

It will be seen from this experiment that a fairly constant ratio of nitrogen of hippuric acid to total nitrogen was obtained; namely, 1 : 9, 1 : 12, 1 : 12. This ratio is nearly double the ratio which has been heretofore obtained. The ratio of the dextrose to nitrogen however was by no means as high as that obtained by Lusk¹; namely, 2.8 : 1. This experiment was repeated several times with the modification that more benzoic acid was given daily (Tables X and XI), as it was thought enough benzoic acid had not been given to combine with the excess of glycocholl formed.

¹ LUSK : *Zeitschrift für Biologie*, 1898, xxxvi, p. 82.

TABLE IX.

Rabbit = 2.1 Kilos.

	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Phlorizin injected subcutaneously.	Hippuric Acid excreted in Grams.	Dextrose excreted in Grams.	Ratio of Dextrose to Total Nitrogen.	Ratio of Nitrogen of Hippuric Acid to Total Nitrogen.
2d day of fast.	0.890			0.044			1:25.1
3d "	1.113	.50 gm. 9 A. M. .50 " 9 P. M.		1.452			1:9.8
4th "	.998	.50 " 9 A. M. .25 " 9 P. M.	.5 gm. 9 A. M. .5 " 4 P. M. .5 " 12 P. M.	1.404	0.813	0.81:1	1:9.1
5th "	1.256	.25 " 9 A. M. .25 " 4 P. M. .25 " 12 P. M.	.5 " 9 A. M. .5 " 4 P. M. .5 " 12 P. M.	1.311	1.475	1.17:1	1:12.2
6th "	1.344	.25 " 9 A. M. .25 " 4 P. M. .25 " 12 P. M.	.7 " 9 A. M. .7 " 4 P. M. .7 " 12 P. M.	1.377	1.528	1.14:1	1:12.4

TABLE X.
Rabbit = 2.4 Kilos.

	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Phlorhizin injected subcutaneously.	Hippuric Acid excreted in Grams.	Dextrose excreted in Grams.	Ratio of Dextrose to Total Nitrogen.	Ratio of Nitrogen of Hippuric Acid to Total Nitrogen.
2d day of fast	0.954			0.044			1 : 280.
3d " "	0.888	1.0 gm. 9 A. M. 0.5 " 6 P. M.		1.733			1 : 6.54
4th " "	0.368	0.5 " 9 A. M. 0.5 " 4 P. M. 0.5 " 12 P. M.	.5 gm. 9 A. M. .5 " 4 P. M. .5 " 12 P. M.	1.056	0.996	2.7 : 1	1 : 4.44

The animal exhibited toxic symptoms on the fifth day, the flow of urine was almost entirely stopped and only about 15 c.c. was collected during the day.

TABLE XI.
Rabbit = 2.4 Kilos.

	Total Nitrogen in Grams.	Benzoic Acid ingested per Os.	Phlorhizin injected subcutaneously.	Hippuric Acid excreted in Grams.	Dextrose excreted in Grams.	Ratio of Dextrose to Total Nitrogen.	Ratio of Nitrogen of Hippuric Acid to Total Nitrogen.
2d day of fast.	.794			.036			1 : 283.
3d " "	.936	1.0 gm. 9 A. M. 0.5 " 6 P. M.		1.612			1 : 7.4
4th " "	.416	0.5 " 9 A. M. 0.5 " 4 P. M. 0.5 " 12 P. M.	.5 gm. 9 A. M. .5 " 4 P. M. .5 " 12 P. M.	1.440	0.770	1.87 : 1	1 : 3.7

Animal died on the fifth day at 6 P. M.; very weak, passage of urine very small.

The results of the experiments given in Tables X and XI serve to show that the two drugs interfered with the action of the kidney, since the rise in nitrogenous metabolism following on the diabetes did not occur, and that the excretion of the urine was retarded so much that no especial conclusions can be drawn from the results.

In closing we would like to express our thanks to Prof. Herbert E. Smith for his kindness in allowing the use of his laboratory throughout these experiments.

CONCLUSIONS.

1. In a fasting rabbit which had been frequently fed with lithium benzoate the amount of glycocoll eliminated through the urine as hippuric acid compared with the total nitrogen indicates that 4.0 grams of glycocoll may be derived from the metabolism of every 100 grams of the body's proteid. The glycocoll excretion runs parallel with the proteid destroyed.

2. Feeding carbohydrates will not increase the formation of glycocoll.

3. After feeding gelatine, it may be estimated that 3.1 grams of glycocoll may be derived from the combined metabolism of gelatine and proteid.

4. After feeding casein, 3.45 grams of glycocoll may be produced from the metabolism of proteid in the organism.

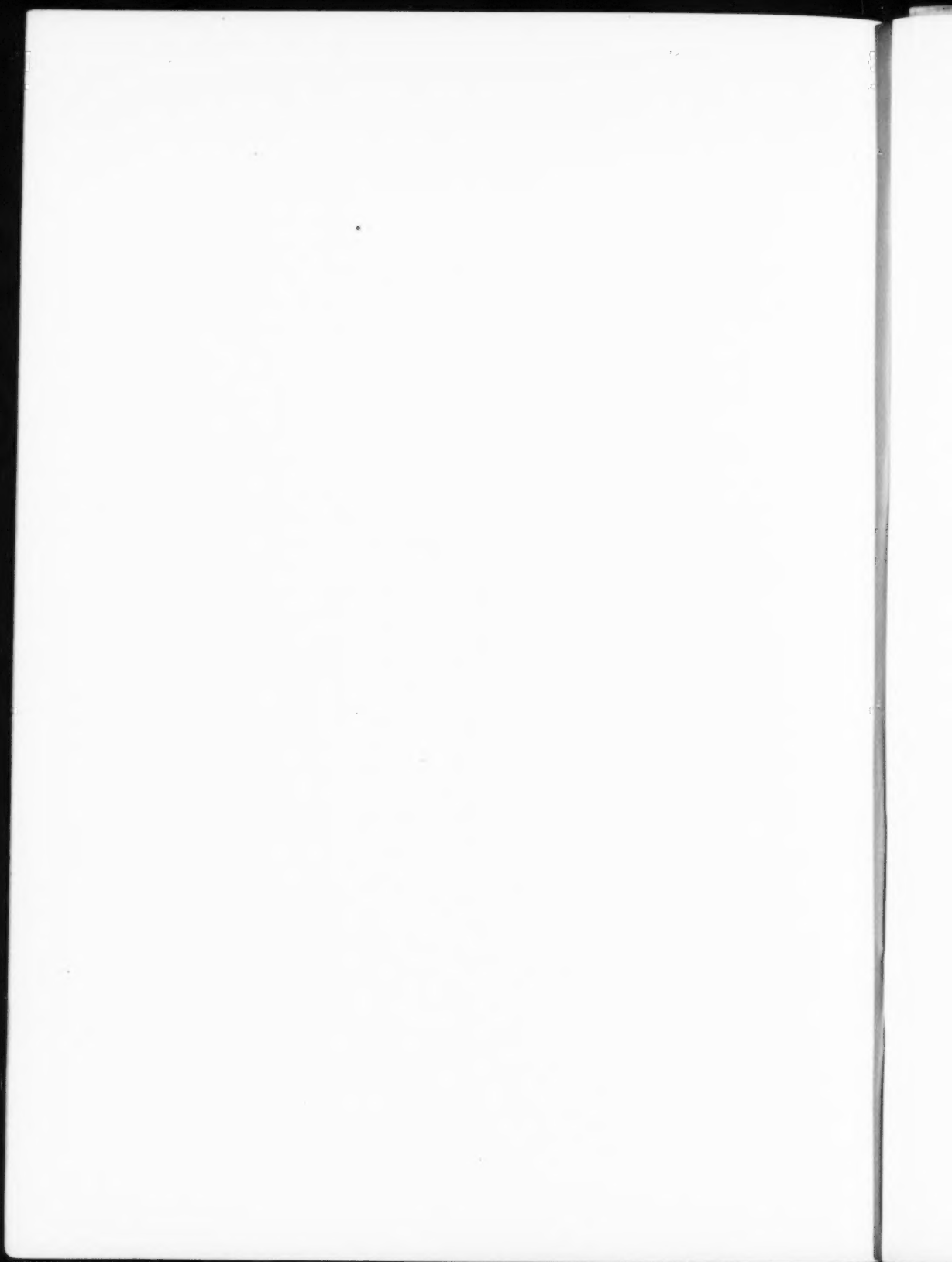
5. It may be fairly concluded that in metabolism the proteid molecule (and gelatine) may yield glycocoll to the extent of at least three to four per cent.

6. In one experiment where the influence of phlorhizin and benzoic acid was combined, fully twice the above production of glycocoll from proteid was indicated, but this could not be confirmed.

PROCEEDINGS OF THE AMERICAN PHYSIO-
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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL
SOCIETY.

ON SOME FEATURES OF NITROGENOUS METABOLISM AFTER
SPLENECTOMY.

BY L. B. MENDEL AND H. C. JACKSON.

[Preliminary communication.]

IN 1891, Horbaczewski published the results of experiments which demonstrated that very considerable quantities of uric acid may be formed in spleen pulp subjected, after previous incipient putrefaction, to the oxidizing action of blood. These observations have recently been verified by Spitzer, who has found that the characteristic uric acid formation is limited to the liver and spleen among various tissues examined. Hypoxanthin and xanthin were found to be transformed readily into uric acid by extracts of the liver and spleen. These facts have given rise to the current hypothesis that the spleen may be regarded as standing in some special relation to the formation of uric acid in the living organism. It seemed desirable to investigate this problem by more direct methods, particularly since there is at present no evidence to indicate that in man and mammals the liver is concerned in uric acid synthesis. We have therefore studied certain features of proteid metabolism in the cat and dog after splenectomy.

The preliminary experiments thus far carried out have indicated that the removal of the spleen is not attended by any diminution in the normal output of uric acid observed after diets of various character. Further, it has been found that the ability of the organism to form allantoin after ingestion of thymus or pancreatic tissue is in no way diminished. Thus from the urine of a spleenless dog to which $1\frac{1}{2}$ kilos fresh sheep's pancreas was fed in three days, no less than 0.85 gram allantoin crystallized out on concentration. A small cat fed with 1 kilo fresh sheep's pancreas in five days yielded 0.65 gram allantoin in a similar manner. After uric acid feeding also, the

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characteristic production of allantoin was observed. These results compare favorably with those obtained by Mendel and Brown in the case of normal cats.

In the course of our investigation we have had occasion to feed fresh salivary glands, and lymphatic glands, such as are frequently found abundantly throughout the pancreatic tissue of sheep. In each case a large rise in the uric acid output has been noted in the case of both normal and spleenless animals; indeed, after ingestion of salivary glands the production of allantoin was very pronounced. So far as we are aware, these observations are new and give further indication of the importance of glandular tissue of this type in uric acid production.

In view of the importance of the liver in nuclein metabolism, we have begun a series of experiments to ascertain the influence of simultaneous suppression of both liver and spleen functions, as in phosphorus poisoning after splenectomy.

SOME BASIC DECOMPOSITION PRODUCTS OF EDESTIN.

By P. A. LEVENE AND L. B. MENDEL.

THE object of this work was to study the basic decomposition products of a proteid of which the individuality and purity were indisputable. Edestin, a proteid which could easily be crystallized, was selected for this purpose.

The pure proteid was heated for 72 hours over an open flame in a flask containing a solution of 20 per cent hydrochloric acid and some stannous chloride. The fluid was then treated in the usual way, and the basic substances precipitated by means of phosphotungstic acid. Out of the latter precipitate the bases, histidin, arginin, and lysin were isolated by methods described in articles which have recently appeared from the laboratory of Professor Kossel.

Histidin was obtained as . . .	$C_6H_9N_3O_2 \cdot 2 HCl$.
Calculated	Cl = 31.14%.
Found	Cl = 31.32%.
Arginin was obtained as . . .	$C_6H_{14}N_4O_2 \cdot HNO_3 + AgNO_3$.
Calculated	Ag = 26.54%.
Found	Ag = 26.86%.
Lysin as	$C_6H_{14}N_2O_2 \cdot C_6H_3N_3O_7$.
Calculated	N = 18.67%.
Found	N = 18.55%.

A PRELIMINARY STUDY OF THE COAGULABLE PROTEIDS
OF CONNECTIVE TISSUES.

BY WILLIAM J. GIES AND A. N. RICHARDS.

THIS investigation was prompted by the belief that there is, perhaps, more metabolic activity in the connective tissues than their "passive mechanical functions" suggest, and, therefore, that an increase of our knowledge of their chemical units will be of some value. Ligament, tendon, and hyaline cartilage are the representative forms of connective tissue we have studied thus far in a preliminary way. Aqueous and magnesium sulphate extracts of the thoroughly clean tissues were made, examination with the spectro-scope showing the absence of hæmoglobin. Cartilage has thus far given entirely negative results. Tendon seems to contain two coagulable proteids; one separates at 54° - 57° C., the other at 73° .

Ligament contains much more coagulable proteid than the other forms. Quantitative determinations with the ligamentum nuchæ of the ox show that that particular form of ligament contains 0.65 per cent of coagulable proteid in the fresh moist tissue and 1.98 per cent in the dry. Proteid precipitates regularly in the various extracts at 42° - 50° , 54° - 58° , 66° - 70° , 74° - 76° , and 83° - 85° C. We do not have sufficient faith in the heat coagulation method to conclude from these results alone that there are as many coagulable proteids in ligament as these temperatures may indicate. We think these results are suggestive rather than conclusive, and expect, by fractional precipitation methods and chemical analysis, to determine definitely the number present. Upon extraction with half saturated lime water ligament yields mucin-like material, which later investigation may show is closely related to the gluco-proteids in tendon.

These results with the ligament suggest that, in the preparation of elastin, due regard must be paid to the fact that the tissue contains a fairly large proportion of soluble and coagulable proteid. Possibly some of the variations in the figures reported for the composition of elastin, and in the nature of its decomposition products, may be due to proteid which had not been removed in its preparation.

Along with this research a study of connective tissue extractives is being made. Ligament has been found to contain an unexpectedly large quantity of creatin, and the concentrated extract yields a fairly heavy, brownish precipitate with silver nitrate in the presence of ammonia. Future results in this connection also may bear directly on the question of metabolism in the connective tissues.

THE GLUCO-PROTEIDS OF WHITE FIBROUS CONNECTIVE TISSUE.

BY WILLIAM J. GIES AND WILLIAM D. CUTTER.

THUS far two series of continuous fractional extractions of ox tendon have been made with half-saturated lime water and the mucins precipitated from each of the extracts analyzed. The semi-cartilaginous character of the sheath in which the divisions of the main shaft of the Achilles tendon move suggested at the outset that possibly the mucin from the sheath may be different from the mucin of the strictly tendinous portion. In the previous work no such discrimination was made, but both parts were extracted together. A comparison of the results for the nitrogen content of the mucins of the first three extracts of both series from the tendon and its sheath show that the nitrogen is lower in the second of each than in the first and third, and highest in the third. The figures range from 11.69 to 13.27 per cent. The sulphur content is highest in the first of each, the figures varying from 1.38 to 2.78 per cent. These results indicate that there are several mucins in white fibrous connective tissue; just how many, our future work may determine.

Further experiments on the glucosazone-like substance obtainable from the reducing bodies gave products melting at 182° C. Thus far it has not been possible to entirely free the crystals from the brownish globules that occur with it, so that probably these figures are still too low.

Before these experiments were started the similarity in the percentage composition of Mörner's chondromucoid and the tendon mucin analyzed by Chittenden and Gies four years ago suggested to us that the two substances are perhaps closely related. This was further emphasized by the fact that the osazone crystals they obtained had the same general appearance as the crystals of glucosazone and, therefore, might have arisen from glucosamin, one of the decomposition products of chondromucoid. Our own results increase the probability that the two substances are nearly identical.

We believe that continued investigation will show that the differences among the mucins, mucoids, and chondroproteids are not as great as their varying physical properties and behavior have suggested, but that each is a combination of proteid with a gluco-sulphonic acid, the characters of each compound, just as in the case of the nucleoproteids, being dependent largely on the proportions of proteid and acid radicals.

THE PREPARATION OF A MUCIN-LIKE SUBSTANCE FROM BONE.

By WILLIAM J. GIES.

YOUNG, in 1892, working under Halliburton's direction, was unable to separate mucin from bone. This negative result has gained general acceptance in spite of the fact that the method employed by Young could hardly have been expected to yield any other. The bone powder and shavings, in quantities ranging from 2.5 to 11 grams, were extracted with 100 to 500 c.c. of lime or baryta water, and, after several days, the filtered solution was treated with acetic acid. Failure to obtain precipitates under these conditions led to the conclusion that bone does not contain mucin. Calling attention to the main defect in this procedure, it is sufficient to suggest that the inorganic substances in bone must necessarily impose a mechanical obstacle to the action of the dilute alkali, and that their removal ought to be the first step in any attempt to get at whatever glucoproteid might be contained in the tissue.

I have prepared a mucin-like substance from the rib and femur of the ox by the following method: The perfectly clean bones were kept in 0.2% to 0.5% HCl. As the inorganic matter dissolved out, the bones were shaved and the shavings accumulated in 0.2% HCl. These were finally run through a meat chopper, then washed free from acid, and extracted in half-saturated lime water. The filtered extract gave a heavy precipitate with 0.2% HCl in excess. 1700 grams of femur shavings yielded 7 grams of this material; 875 grams of rib shavings gave 3.5 grams. This substance appears to be mucin, though it may be chondromucoid or a relative of each. It dissolves easily in 0.1% Na_2CO_3 . It is acid to litmus. It gives the proteid color reactions, yields a reducing substance, and contains ethereal sulphuric acid. The nitrogen and sulphur content of the unpurified substance approximates that of tendon mucin and chondromucoid. The filtrate from the mucin precipitate contains a substance which has many of the qualities of chondroitin sulphuric acid. A careful investigation of the composition and character of the mucin-like substance, and the body supposed to be chondroitin sulphuric acid, is now being made. The general method employed for their detection and separation promises, also, to yield material well suited for the studies we shall make of bone gelatine and the various organic bone constituents.

It is evident from these results that ordinary compact bone, just like the other forms of connective tissue, does contain mucin substance, and, further, that in the process of ossification the connective tissue matrix is not completely removed.

ON MUCIN.

By P. A. LEVENE.

ALL mucins are considered at present compounds of proteids with carbohydrates. This supposition, however, does not explain the acid properties of mucins. It was, therefore, the object of this work to ascertain whether the molecule of mucin contained in itself an acid radical. Thus far the most work was done on tendo-mucin. From the latter was obtained an acid substance with the following properties: It was nitrogenous (but gave none of the proteid tests), did not contain any phosphorus, contained sulphur. A solution of the substance precipitated Witte's peptone on addition of some acetic acid. Heated with mineral acids, the substance was rendered capable of reducing an alkaline copper solution. Further, it was established that the sulphur was combined in the substance in the form of an ethereal sulphuric acid. There could also be obtained from the acid a substance which gave the barium test peculiar to chondrosin.

Thus far the substance seemed similar to chondroitin sulphuric acid.

However, the analysis of the barium and copper salts gave results deviating a little from those expected from chondroitin sulphuric acid. An attempt to ascertain the causes of these deviations is now in progress.

The mucin of the submaxillary gland as well as colloid of a colloidal carcinoma were examined in the same direction, and both proved to contain an ethereal sulphuric acid, with the same properties as that obtained from tendo-mucin. The presence of chondrosin in their molecules is thus far not established, because of lack of material. Further investigation into the nature of the latter two substances is in progress.

CERTAIN IMPROVEMENTS IN THE TECHNIQUE OF
ERGOGRAPHIC WORK.

BY T. HOUGH.

THE paper was a description of an ergograph in use in the Biological Laboratory of the Massachusetts Institute of Technology. Attention was called to the importance of attaching the weight or spring at a constant distance from the joint which serves as the axis of movement. This was attained by the use of a double splint attached to the distal phalanges of the middle finger, one of these splints carrying an adjustable hook which was made fast at a constant distance (determined by a T square) from the joint; the muscle thus works with the same leverage at all times. Attention was also called to the fact that, where the movement used is flexion of the finger, the first phalanx must be made fast so that the axis of movement shall be the joint between the first and second phalanges; this is necessary because the first phalanx is flexed by the lumbrical muscles in the palm of the hand, while the other phalanges are flexed by the flexor muscles in the forearm; flexion of the entire finger must, therefore, involve the co-ordinated use of several muscles, and hence is objectionable in the study of simple fatigue. It was also shown that the weight and spring both have advantages for ergographic work, and the choice of the one or the other must be determined by the nature of the problem to be studied. Finally, certain differences in the curves of trained and untrained muscles were commented upon as affording some clew as to the relations of fatigue to muscular soreness.

ON THE TOXICOLOGY OF POTASSIUM CHLORATE, WITH A
DEMONSTRATION OF THE EFFECTS OF INTRACEREBRAL
INJECTIONS.

BY S. J. MELTZER.

It seems now generally accepted that death from potassium chlorate poisoning is due to the formation of methæmoglobin and the destruction of the red blood cells within the vascular system of the living animal. This explanation is inadequate. It is a well established fact that in rabbits poisoned by potassium chlorate, neither

chemical nor morphological changes take place in the blood of the living animal. Dogs occasionally die before any changes in the blood take place. The formation of methæmoglobin within the blood is comparatively harmless (acetamid, phenacetine, etc.); and we also know that the blood manages to get rid of the debris of the broken down red corpuscles. It is therefore justifiable to assume that potassium chlorate may cause death by some means other than the changes in the blood.

The author observed that after intraperitoneal injections of potassium chlorate rabbits died in a comparatively short time under convulsions preceded by a gradual paralysis of the inspiratory muscles. This observation in conjunction with clinical and experimental experiences of other writers led to a study of the effects of intracerebral injections of this salt. The injection of 3 minims of a 5% solution of KClO_3 into the brain of rabbits causes at once a long series of convulsions, forced movements, opisthotonus, co-ordinated movements, inco-ordinated clonic and tonic convulsions, etc. This state of excitation gradually gives way to a comatose state which not many animals survive. Stronger solutions cause a more stormy scene, but of shorter duration; the animal succumbs in fifteen to twenty minutes. When 3 minims of only a 1% solution are injected the animal runs about incessantly for an hour and longer and then falls into a paretic state lasting for hours; but the animal recovers.

These experiments show potassium chlorate to be a strong poison to nerve cells; they are first excited, then paralyzed by it. It is possible that in poisoning through the blood the salt reaches the vital centres in a concentration sufficient to excite and paralyze them.

The author demonstrated on a rabbit the described effects of potassium chlorate, and also on another rabbit the opposite effect of intracerebral injection of magnesium sulphate: without preceding convulsions the rabbit became paralyzed in a short time. Finally by the intracerebral injection of 0.8% sodium chlorate the harmlessness of the injections as such was shown.

THE USE OF EXCISED MAMMALIAN MUSCLES FOR PURPOSES
OF DEMONSTRATION.

BY JOHN G. CURTIS.

THE author has found that if a cat have been killed by the hypodermic injection of 0.03 gram of curare, its muscles can be excised, covered, until wanted, with filter-paper wet with normal saline solution, and employed without a moist chamber, and at the temperature of the room, for class purposes, at any time during the first two or even three hours after the death of the animal. No greater precautions are needed than in the case of frog's muscle, and the time spent in making two companion preparations from the same animal need not be more than 15 or 20 minutes.

The conjoined gastrocnemius and soleus of the cat may be used for demonstrations of muscular work, and may lift a weight of 2 kilograms, hung directly from the tendon, to a height of 1 centimetre. Where much shortening of the muscle is desired, the conjoined tibialis anticus and extensor longus digitorum pedis may give good results. The change of form of this preparation in contraction is easily visible at a distance, and by its aid, and that of a drum revolved by hand, muscle-curves of great amplitude may be shown. For this purpose the muscle should be attached 10 centimetres from the axle of a wooden lever 53.5 centimetres long. The lever should be shaped like a tapering beam 3 millimetres thick, the vertical dimension of its recording end being 5 millimetres. The edges and corners of this end should be very slightly rounded, and a strip of velvet should be glued to the wood in such a manner as to cover the end and about 1 centimetre of each lateral surface. By means of such a lever, isotonicallly weighted with 250 grams, a curve of tetanus may be written with a plateau 11.5 centimetres above the base-line, the width of the even line traced by the lever being 5 millimetres. Very beautiful curves may be produced by this method for the demonstration to a large audience of superposition, of the genesis of tetanus, and of fatigue.

THE EFFECTS OF CHANGES IN EXTERNAL TEMPERATURE
UPON THE CUTANEOUS CIRCULATION.

By T. HOUGH.

IN this work a study was made of the effects of changes in external temperature upon the capillary pressure in the skin and upon the size of the veins leading off from the capillary regions studied, the latter being taken as a rough indication of the amount of cutaneous blood flow. It was found that low temperatures (from 5° to 15° C.) produce a diminished blood flow accompanied by increased capillary pressure. Higher temperatures (25° to 35° C.) produce an increased blood flow without necessarily changing capillary pressure. Attention was called to the fact that capillary pressure could not in this way be independent of the amount of blood flowing through the skin if the only determining factor is the constriction of the arterioles. It was furthermore pointed out that the facts are all explicable on the assumption that the muscular coats of the small veins play an important part in the regulation of capillary pressure; the high pressure observed with the low temperatures would thus be explained by supposing that the arterial constriction is accompanied by venous constriction, and the maintenance of a constant capillary pressure with higher temperatures would be the result of venous dilation which permits easier egress of blood. Fairly constant capillary pressure would thus be consistent with considerable variations in the amount of blood flowing through the organ.

SOME CHEMICAL CHANGES IN THE DEVELOPING EGG.

By P. A. LEVENE.

THIS work gives the results of an attempt to elucidate the chemical process of construction of animal tissue. Thus far the investigation has been limited to the distribution of nitrogen in the different nitrogenous compounds of the developing egg of different ages. All the nitrogenous substances produced on decomposition of proteids may be classified in two distinct groups: Those of acid nature, like the monoamido-acid, and those of basic nature. The following table demonstrates to some extent the part the same substances play in tissue construction:

	Unfertilized eggs. Per cent.	24 hours after fertilization. Per cent.	10 days after fertilization. Per cent.	19 days after fertilization. Per cent.
Nitrogen in Monoamido- compounds	21.10	21.37	22.72	0.
Nitrogen in form of bases	12.07	25.10	12.48	28.25
Nitrogen in form of proteids	66.0	53.57	64.79	71.84

It has also been found that the quantity of the xanthin bases and of nucleo-compounds increases with the growth of the egg embryo. The importance of mineral salts for the formation of tissues was demonstrated by the increasing quantity of mineral substance in the egg in the course of its growth.

ABSTRACT OF PAPER ON THE NUTRITIVE VALUE OF ALCOHOL.

By W. O. ATWATER.

THIS epitomized the results of the late experiments with men in the respiration calorimeter at Wesleyan University, in which the nutritive action of alcohol was compared with that of fats, sugar, and starch of ordinary food. Experiments in which the subject had ordinary food were compared with similar experiments in which alcohol formed part of the diet. The individual experiments continued from two to four days each. In general, the diets whose effects were thus compared contained the same or nearly the same quantities of protein and energy, the difference being that a certain amount of the fats, starch, and sugar (or sugar alone) of the diet in the one case was replaced by an isodynamic amount of alcohol in the other. In a majority of the experiments, pure ethyl alcohol was used, but in some, whiskey and brandy were employed. The quantity of absolute alcohol in each case was about $72\frac{1}{2}$ grams per day, the potential energy being equivalent to about 500 calories. In some of the experiments, the man was at rest, in others, he was engaged in active muscular work. The diet was such as to provide more or less nearly for nitrogen and carbon equilibrium. The alcohol supplied about one fifth of the total energy in the rest experiments and about one seventh

in the work experiments. The results show the balance of income and outgo of nitrogen, carbon, and energy in the body. The alcohol excreted by the kidneys, lungs, and skin was collected and measured and found to be, in general, under rather than over 2 per cent of the whole amount ingested, thus indicating that 98 per cent was oxidized in the body. The income and outgo of energy agreed very closely, thus showing that the potential energy of the alcohol oxidized was transformed into measurable kinetic energy (heat and external muscular work). There were some variations in the nitrogen and carbon balance in the different experiments, but these were not such as to indicate any marked difference between the fats and carbohydrates on the one hand and the alcohol on the other in their function of protecting food and body material from consumption. The inference is that the alcohol actually served the body for fuel as did the fats and carbohydrates. The question as to whether the fuel values of alcohol, sugars, starch, and fats correspond with their respective heats of combustion, or, in other words, whether and to what extent their values for furnishing energy to the body in different kinetic forms and for protecting body and food material from consumption, accord with their isodynamic values can be decided only by a large amount of detailed research.

ON THE FUNCTIONS OF BILE AS A SOLVENT.

By B. MOORE.

THE bile has a twofold function as a solvent. In the first place, it effects the secretion of certain waste metabolic products from the liver which are insoluble in water, such as lecithin and cholestearin. In the second place, it dissolves, and so prepares for absorption from the intestine, certain products of fat digestion, such as the insoluble soaps and free fatty acids.

The solvent properties of the bile are due to the bile salts, but the dissolving power is markedly increased by the simultaneous presence in the solution of lecithin.

Cholestearin is but feebly soluble in solutions of bile salts, and this furnishes an explanation of the fact that gallstones commonly consist of almost pure cholestearin.

Lecithin, on the other hand, is very soluble in solutions of bile

salts, though practically insoluble in water. Further, the solubility of cholestearin is increased by the presence of lecithin in the solution.

The calcium and magnesium soaps have only a very slight solubility in bile salts alone, but are somewhat more soluble when lecithin in addition is present.

The sodium soaps are considerably more soluble in bile salt solutions than in water, and bile accordingly aids in the solution of these soaps in the normal process of fat digestion.

The physical character of the solutions of the sodium soaps is also altered in the presence of bile, the solution becomes less viscid when bile is present, and on cooling the soaps are precipitated in a granular form and not as a jelly as in the case of solution in water alone.

ON THE EFFECTS OF INTRAVENOUS INJECTION OF MINIMAL DOSES OF SUPRARENAL EXTRACT UPON THE ARTERIAL BLOOD PRESSURE.

BY B. MOORE AND C. PURINTON.

CURVES were exhibited demonstrating the effects obtained in the dog by injection of extracts of suprarenal medulla in doses varying from 0.245 to 24 *millionths* of a gram per kilogram of body weight.

The results obtained show in the first place that the crude medullary extract is many times more powerful physiologically than any of the so-called pure products which have hitherto been obtained by other observers.

In the opinion of the authors, any activity possessed by these so-called isolated salts of the active substance may as easily be explained by slight contamination with the unaltered active principle, as by the supposition that they consist of chemical individuals containing the active material in an altered and comparatively inactive form.

In the second place, the effects obtained by minimal injections show that as the dose is diminished the pure rise first obtained, which has hitherto been recognized as the characteristic result of suprarenal injection, is replaced by a rise which is succeeded by a greater fall, and with a still smaller dose a marked fall is the only result obtained.

The authors believe that this fall with minute doses is to be attrib-

uted to an opposite action of the active principle in small doses to that which it possesses with the usual doses, rather than to the action of a second blood pressure reducing substance. For, if two substances be present in the extract with opposite actions on blood pressure these would be equally diluted as the dose was reduced, and the power of both would most probably be diminished *pari passu*. Hence it is difficult to understand how an opposite effect could be obtained with minimal doses.

ON THE CHROMOGEN OF THE SUPRARENAL MEDULLA, AND
ON ITS RELATIONSHIP TO THE ACTIVE SUBSTANCE.

BY B. MOORE AND C. PURINTON.

A NEW color reaction of the chromogen was described which is obtained by the addition of dilute ferric chloride after excess of zinc acetate to extracts of suprarenal medulla. Here a deep violet color is obtained instead of the usual olive green given by ferric chloride alone. The color is evanescent, and in strong solutions leaves a violet colored precipitate.

A method was also pointed out for separating the active substance from a number of impurities. This consisted in boiling the separated medulla with very dilute acetic acid for a few minutes to coagulate proteids, adding zinc acetate to the warm filtrate, giving a copious precipitate of inactive substances, which are thus separated from the active substance. The filtrate is freed of zinc acetate by passing sulphuretted hydrogen, and is still found to be as active as the original solution, although it contains only about one fourth the quantity of organic solid.

On standing, this filtrate loses its activity slowly, and in the end is found to be inactive at a period when it still shows marked color reactions. This is a further proof of the point which is often ignored, that the activity does not depend upon the integrity of the chromogenic group, although that may form a portion of the still more complex molecule which possesses the physiological action. It follows that the presence of chromogen, as shown by color tests, must not be taken alone to indicate the presence of active material.

It was further pointed out that the use of alkalis should be avoided in any method devised for the isolation of the active sub-

stance, since the activity is thereby rapidly destroyed. This point has been neglected in most methods hitherto described, and such neglect is responsible for the almost complete destruction of activity.

ON THE PHENYLCARBAMIC ESTERS OF EPINEPHRIN.

By JOHN J. ABEL.

IN the preparation of these esters the following methods are employed. The benzoyl compound of epinephrin is prepared from aqueous extracts of the gland. From this compound epinephrin picrate is prepared, and this salt is in turn converted into epinephrin bisulphate. The dry bisulphate is treated with an excess of phenylisocyanate ($\text{CO.N.C}_6\text{H}_5$). A lively reaction begins at 70° , but the temperature is raised by means of an oil bath to 144° or even to 166° . The resulting ester is washed with benzol and ether, dissolved in methyl alcohol, precipitated with a mixture of ether, benzol, and petroleum ether, and after a second solution and precipitation it is converted into a picrate. This salt is converted into a sulphate, which is first subjected to a purifying process and is then again converted into a picrate. This salt is then turned into a sulphate which is dissolved in methyl alcohol and precipitated with water. The preliminary analyses for carbon, hydrogen, and sulphuric acid show that this salt is the sulphate of the phenylcarbamic di-ester of epinephrin, $[\text{C}_{17}\text{H}_{13}\text{NO}_4(\text{CO.NH.C}_6\text{H}_5)]_2 \cdot \text{H}_2\text{SO}_4$. The general formula for the formation of esters of phenylcarbamic acid is $(\text{R})\text{OH} + \text{CO.N.C}_6\text{H}_5 = (\text{R})\text{O.CO.NH.C}_6\text{H}_5$.

Although two of the hydroxyl groups in epinephrin have entered into combination with phenylisocyanate to form the ester just described, it is found that physiological activity is retained. Solutions in very dilute alcohol still raise the arterial pressure. Quantitative measurements of its physiological activity have not yet been made. No by-product possessing the power to raise the blood pressure is formed in the process of esterification. Saponification under pressure, in very dilute alcohol, leads to the original bisulphate of epinephrin. On treating the di-ester in a sealed tube with phenylisocyanate, a second ester is formed which differs in some of its reactions from the former, and which is probably the phenylcarbamic tri-ester of epinephrin. These esters furnish additional

evidence in support of the opinion that epinephrin is a chemical individual and not a mixture of substances. If the original bisulphate of epinephrin had been much contaminated, it is difficult to see how an ester of the above formula could have been obtained from it.

NOTE ON A BLOOD-PRESSURE LOWERING BODY IN THE SUPRARENAL GLAND.

By R. HUNT.

THE material examined was an aqueous extract of the suprarenal glands from which epinephrin had been removed by Dr. Abel by means of benzoyl chloride and which no longer gave a rise of blood pressure on intravenous injection. This solution was freed from benzoic acid, evaporated to dryness, and taken up in alcohol; to this alcoholic extract an excess of a solution of mercuric chloride in alcohol was added. A precipitate, almost insoluble in water, was thus formed; this was suspended in water and decomposed by hydrogen sulphide. The filtrate was evaporated to dryness, taken up in alcohol, and an alcoholic solution of platinum chloride added. The crude precipitate was dissolved as far as possible in water, and to the solution hot alcohol added until the strength of the latter was about forty per cent. As the solution cooled octahedra separated out; these were re-crystallized two or three times and were found to have the following properties: they were readily soluble in cold water, insoluble in alcohol, and gave the odor of trimethylamine on heating. When these crystals were decomposed by hydrogen sulphide and the filtrate injected into an animal a marked fall of blood pressure, and usually a slowing of the heart, resulted; after the administration of atropine there was either no effect or a rise of blood pressure. Thus in both its chemical and physiological properties the substance isolated agrees with choline; one analysis of the platinum compound which was entirely free from impurities gave 31.63 per cent of platinum, whereas 31.64 per cent is required by the platinum compound of choline.

Preliminary physiological experiments with extracts of sympathetic ganglia and the brain indicate that the blood-pressure lowering bodies of these substances are probably identical with that of the suprarenal gland; the solubilities of these bodies seem also to be the same.

Some evidence was obtained that some other blood pressure lowering body (not precipitated by mercuric chloride) is also present in aqueous extracts of the suprarenals. These blood-pressure lowering bodies are not necessarily decomposition products of lecithin, jecorin, etc., as they are present in dialysates of the gland in which epinephrin has been destroyed by animal charcoal; also in glands from which lecithin and jecorin have been removed by long continued extraction with ether.

THE INFLUENCE OF PROTOPLASMIC POISONS ON THE FORMATION OF LYMPH.

BY WILLIAM J. GIES AND LEON ASHER.

THE work reported upon here very briefly was done in the Physiological Institute at Bern. An attempt was made in this investigation to ascertain, as far as possible, the changes which may occur in lymph after the administration of protoplasmic poisons, by studying the influence of such poisons on the phenomena usually produced by well known lymphagogues. In this way we attempted to distinguish between the so called physiological and the physical factors participating in the production of lymph. Our experiments were on dogs and with quinine and arsenic. The usual methods of lymph collection and analysis afford the data for our conclusions.

Quinine did not interfere with the usual influence of dextrose, although it did suppress the action of leech extract. Our results with dextrose, therefore, indicate that the increase in the quantity of lymph following its injection in large quantity is due mainly to physical factors. In the case of leech extract, on the other hand, we conclude there has been an interference with the action of the physiological factors that appear to be responsible for the changes usually brought about by this lymphagogue.

That the increase in the amount of lymph after large quantities of dextrose have been injected is not due specifically to increased capillary pressure, as is held by Cohnstein and Starling, was shown in one of our experiments in no uncertain way. After an injection of 1 gram of quinine, 25 grams of dextrose and 0.5 gram more of quinine followed ten minutes later, and 35 c.c. of blood was drawn off. Almost immediately the usual effect of dextrose became evident. In a few

minutes, however, the dog died, yet, for more than three hours thereafter, the flow continued, and that, too, without artificial respiration or any mechanical assistance whatsoever. The rate of flow gradually increased for more than an hour, when it slowly fell back to, and below, the rate of the first period. During the three and a half hours of the experiment the total flow of lymph was 140 c.c. During the first half hour, when the normal conditions prevailed, the flow was only 12.8 c.c. The amount of total solids at the start was 5.02%, at the end 5.9%. The sugar rose from 0.19% to 2.2%. This experiment seems to emphasize Heidenhain's view that the increase of lymph following injections of large quantities of dextrose is due to changes of osmotic pressure in the tissue spaces.

Following injections of arsenic, which is said to very greatly increase the permeability of the blood vessels, especially those of the portal system, there was little in the flow and character of the lymph resembling the usual effect of lymphagogues. We conclude, therefore, that Starling's hypothesis does not fully account for the action of lymphagogues, and that the mechanical theory of lymph formation fails as long as it does not explain the most striking phenomena of the process — those following the injection of Heidenhain's lymphagogues or Asher's "liver stimulants." The physiological theories of Heidenhain and of Asher and Barbèra would explain them.

THE PHYSIOLOGICAL ACTION OF TELLURIUM COMPOUNDS.

BY WILLIAM J. GIES AND L. D. MEAD.

OUR work with tellurium compounds was begun at the suggestion of Dr. Victor Lenher, who very kindly furnished us with an abundant supply of chemically pure tellurium preparations. In view of the use of potassium and sodium tellurates as antihydrotics, to reduce the night sweats of pulmonary consumption, we have determined the influence of small quantities of tellurium compounds on the nutritional processes. We find that quantities of tellurous oxide, sodium tellurite, and tellurium tartrate, not exceeding 0.1 gram daily in two doses, do not materially alter proteid metabolism in dogs brought to a state of nitrogenous equilibrium, even when the dosage is continued for a week. After the administration of these non-toxic amounts the

faeces were fairly constant in elimination, quantity, and character. There was no appreciable effect on the elimination of water. Digestion did not appear to be materially hindered. Tellurium was eliminated in the urine and the odor of methyl telluride in the expired air was very pronounced.

Larger doses, however, 0.2 to 0.5 gram at a time, cause violent vomiting and induce disintegration of the gastric mucous membrane. Our experiments on a dog with gastric fistula show that there is a very decided interference with the secretion of hydrochloric acid after the administration of tellurium in these quantities and, also, that regurgitation of bile is one of the consequences. The action of pepsin and trypsin outside the body is not materially influenced by quantities of tellurium tartrate and sodium tellurite under one per cent.

Tellurium is eliminated in the breath, urine, and faeces of the dog. Reduction to the metallic state occurs when tellurium compounds come in contact with the tissue cells, though tellurium itself is soluble in the body juices and is distributed to the various organs. Two days after subcutaneous injection of a little more than 1 gram of the tartrate, 38 milligrams of tellurium were recovered from the tissue about the point of injection, 12 from the liver, 9 from the kidneys, 7 from the bile, and 3 from the brain. Additional experiments will be made with sodium and potassium tellurates.

THE REACTION TIME OF INHIBITION.

BY ALLEN CLEGHORN AND COLIN C. STEWART.

FICK has stated that the application of an electrical stimulus to a muscle, voluntarily contracted to the fullest extent, will produce relaxation.

It was found that if the arm be fixed in Mosso's ergograph, and the middle finger be held contracted, the contraction can be inhibited by strong sensory stimuli applied to the other arm or to any part of the body. Electrical stimuli, sound, and light were used.

The time between the sending in of the sensory impulse and the resulting interference with the contraction is taken as the inhibition time. It is measured by recording the contraction, and the relaxation following the sensory stimulus, on a rapidly moving drum, by means of a lever to which is attached an electro-magnet. The magnet is

connected in such a way as to short circuit the primary current, the breaking of which gives the induced current used as the stimulus. The tracing and a time record in hundredths of a second are thus made so compact that twenty-five records can easily be taken on a single drum.

The result of the measurement of several hundred such records from fifteen subjects showed the inhibition time to be much slower than their reaction time taken with the same apparatus, the averages being 19.63 as compared with 13.34 for the reaction time.

THE EFFECT OF STIMULATING VARIOUS PORTIONS OF THE CORTEX CEREBRI, CAUDATE NUCLEUS, AND DURA MATER UPON BLOOD PRESSURE.

BY W. H. HOWELL AND M. F. AUSTIN.

THE paper gave an account of a number of experiments made upon dogs in which various regions of the cortex cerebri, the caudate nucleus, and dura mater were stimulated with induction currents, and the effect upon the blood pressure determined with regard to the cortex cerebri. It was shown that certain regions when stimulated give a definite vasomotor response, which may be either pressor or depressor in character. Most of the sigmoid region, anterior and posterior sigmoid gyri, gave a constant vasomotor effect. When the anæsthetic used was morphia and ether, the effect was, as a rule, to cause a fall of blood pressure. With morphia and curare, however, the general result was an increase in blood pressure. The area from which these vasomotor effects were obtained most easily was in the anterior sigmoid gyrus, corresponding to the neck area as given by Fritsch and Hitzig. Other areas from which similar results were obtained were in the coronal gyrus, the facial or eye region, and the extreme anterior portion of the prorean gyrus. As these areas all lie within the motor region, it seems possible that the motor innervation proceeding from them may be accompanied by a simultaneous effect upon the vasomotor centre.

Stimulation of the portion of the caudate nucleus projecting into the lateral ventricle caused, under favorable conditions, a marked rise of blood pressure, together with an inhibition of the respiratory movements.

Most interesting vasomotor effects were obtained from stimulation of the dura mater. It was found that this membrane, particularly on its inner face, is very sensitive to mechanical stimulation, provided the ether anaesthesia is not too deep. Pinching, cutting, or even gentle pressure with a moist sponge may cause a very marked change in blood pressure, together with greatly increased respiratory movements. When the animal was anaesthetized with morphia and ether, the usual vasomotor effect was a fall in blood pressure. After the injection of curare, however, stimulation under the same conditions gave usually a rise in blood pressure. To gentle mechanical stimulation, at least, the dura seems more irritable than the other sensory membranes of the body.

THE RELATION OF THE DEPRESSOR NERVE TO THE VASOMOTOR CENTRE.

BY W. T. PORTER AND H. G. BEYER.

It is conceivable that an afferent nerve, such as the depressor, should make connection, not with all the cells of the centre in which it ends, but with certain cells only. Thus it has been thought that the depressor nerves are connected with the vasoconstrictor fibres in the splanchnic nerves in a way differing in degree, if not in kind, from the connection of the depressor with other vasomotor fibres. If the splanchnic nerves are severed and their peripheral ends stimulated until the blood-pressure returns to its normal level, and if then the central ends of the depressor nerves are stimulated, the fall in blood-pressure is nearly and sometimes quite as great as that obtained by stimulating the depressor nerves after the splanchnic nerves have been exposed but are still intact. The difference usually observed seems probably due to the shock of the abdominal operation. The authors find no evidence to warrant the opinion that the depressor nerves have special connections with the cells in the vasomotor centre associated with the splanchnic fibres. This afferent nerve appears to communicate in the same manner and in like degree with all the cells in the centre in which it ends. It is probable therefore that other nerves afferent to this centre have likewise an impartial connection.

THE VASOMOTOR NERVES OF THE HEART.

BY W. T. PORTER AND H. G. BEYER.

IN 1895 it was observed by W. T. Porter that the outflow from the coronary vessels of an isolated heart, fed with defibrinated blood at a constant pressure, was markedly lessened when the peripheral end of the vagus nerve was stimulated. The conclusion was drawn that the vagus nerve contained vasoconstrictor fibres to the coronary vessels. Some months later it was discovered that each contraction of the ventricle empties the intramural vessels, thereby favoring their refilling in diastole, and thus augmenting the volume of the coronary circulation. This discovery renders the evidence for vasoconstrictor fibres in the vagus uncertain. For the diminished outflow from the coronary vessels during vagus stimulation may be due to the lessened force with which the ventricle empties the intramural vessels. The authors have endeavored to find a stimulus to the vagus which will act on the vasoconstrictor fibres but not on the inhibitory fibres. In the effort to separate the two sets of fibres, cold, fatigue, different rates of stimulation, degeneration, atropine and other drugs, have been employed, but thus far without success. The presence in the vagus of vasoconstrictor fibres for the coronary vessels must be regarded therefore as probable rather than quite certain.

SPINAL RESPIRATION.

BY W. T. PORTER AND W. MÜHLBERG.

THE fact that the respiratory movements of the diaphragm cease when the phrenic cells are separated from the general respiratory centre by a cross-section of the spinal cord is said by the advocates of spinal respiration to be the result of the inhibition of the phrenic cells by the violence of the section and the continued stimulation proceeding from the wound. Evidence of the incorrectness of this opinion was given by one of us in 1895.¹ It was then shown that the phrenic cells alleged to be inhibited by a hemisection of the cord became instantly active when the phrenic nerve of the side opposite to the hemisection was severed. It was shown further that the

¹ W. T. PORTER: *Journal of physiology*, 1895, xvii, pp. 455-485.

phrenic cells were still passive on the side of the hemisection seven hours after the separation of the fibres connecting them with the bulb, though they immediately resumed their function on section of the opposite phrenic nerve. In response to a desire of Professor Jacques Loeb to know whether the phrenic cells would discharge respiratory impulses if the animal were kept alive longer than seven hours after the isolation of the cells from the bulb, the authors have in a great number of rabbits and cats separated the right and left sets of phrenic nuclei by completely dividing the spinal cord in the median line from the 2d cervical to the 1st dorsal vertebrae and then have isolated completely the phrenic cells of one side by a hemisection on that side at the level of the 2d cervical vertebra. Animals thus treated have been kept alive as long as eight days, at the end of which time direct inspection of the diaphragm showed an entire absence of contraction on the side of the hemisection. The phrenic cells on the operated side were functional, nevertheless, for reflex contractions could still be obtained from them. These reflex movements did not resemble the rhythmic succession so characteristic of true respiration.

MAMMALIAN SMOOTH MUSCLE.

BY COLIN C. STEWART.

THE method consists in the suspension of the cat's bladder between a fixed point and a direct recording lever. This may be done either in the living anaesthetized animal or in the moist chamber of a water jacket, the former method obviating the errors introduced by fatigue, though failing to maintain normal temperature.

Spontaneous contractions are of frequent occurrence, some of the tracings obtained showing compound rhythms of the kind shown by Bowditch before the British Association at the Toronto meeting. These contractions, once begun, have in many cases been continuous during periods of twenty-four, twenty-eight, and thirty hours at ordinary room temperatures.

Contractions have been obtained in response to electrical stimuli from a bladder, kept meanwhile in an ice box, ninety-six hours after excision.

With the rise in temperature from 10° to 55° C. the form of the

contraction varies considerably. The latent period shortens, and the time of the rise is greatly decreased. The height of the contraction is greatest at temperatures slightly above the normal. Heat rigor appears at from 53° to 58° C.

Single contractions at body temperature are fused into a typical tetanic contraction when the stimuli are applied at intervals slightly less than two seconds. With longer intervals summation is obtained until the interval is increased to from twelve to twenty seconds, variations occurring with the condition of the bladder with respect to fatigue.

The bladder responds to ordinary induction shocks, and to both the make and the break of the constant current, but more to the make. None of the results so far has shown any antagonism between the make and the break shocks in their influence on the bladder. There is often evidence of an influence of the flow of the current in sustaining the contraction produced by the make.

The results obtained with the bladder confirm Woodworth's finding that an appreciable interval must occur between the make and the break, and between the break and the make, to produce any effect from the double stimulus, and that within limits the effect varies with the interval.

A FURTHER CONTRIBUTION TO THE PHARMACOLOGY OF CHLORETONE.¹

BY T. B. ALDRICH AND E. M. HOUGHTON.

WHEN chloretone is administered to animals per stomach, it produces all degrees of hypnosis to complete anæsthesia, lasting from a few hours to several days, depending on the amount of the substance entering the system, the animal finally recovering in excellent condition, if the dose is not too large.

The drug is quickly absorbed from the alimentary tract, especially when in solution, and can readily be obtained from the blood in crystalline form. The spectroscope fails to show any effect of the drug on the hæmoglobin of the blood in vitro, or in the animal body. The pulse rate is slightly lessened, but the action of the heart, under the influence of chloretone, remains excellent until the organism begins

¹See Journal of the American Medical Association, Sept. 23, 1899.

to suffer from a lack of oxygen. Frogs are very quickly overcome by the action of the drug. The local application of aqueous solutions of chloretone to the laid-bare frog's heart produces slowing of the rate, and a more complete contraction, reminding one of the digitalis heart.

Kymographic tracings taken from the carotid artery of dogs or rabbits show that the blood pressure remains practically unaffected.

The amplitude of the contractions of the ventricle as recorded by the myocardiograph likewise remains unchanged for many hours.

Chloretone possesses, also, local anaesthetic properties in a marked degree.

The action of the drug upon the central nervous system is essentially the same as that of the other anaesthetics and hypnotics of the fatty acid series, differing from most of the members of this group in not depressing the circulatory system.

Quite large quantities of chloretone can be obtained from the brain substance after as much of the blood has been removed as possible from that organ by perfusion.

Culture experiments with various kinds of bacteria also prove that the drug possesses considerable germicidal and antiseptic properties.

We would naturally expect, since chloretone is volatile at body temperature and since it circulates in the blood, that it would be eliminated by the lungs. Carefully conducted experiments thus far have failed, however, to detect it in the expired air. However, small amounts of acetone are apparently eliminated in this way.

The chlorides in the urine of a dog are markedly increased after the administration of chloretone. If the administration in small amounts is kept up for several weeks, small quantities of chloretone also appear in the urine.

That chloretone does not lower the vitality of animal cells is evidenced by the fact that wounds to which it has been applied, as a dressing, heal quickly by first intention, and that ciliated epithelia and spermatozoa remain active for many hours when bathed with a saturated aqueous solution.

Chloretone is nearly an ideal anaesthetic for use in experimental surgery, physiology, pharmacology, etc., when given in doses of 0.2 to 0.25 gram per kilo of body weight in warm saturated aqueous solution, the animal becoming completely anaesthetized in a very short time.

INFUSION AFTER SEVERE HEMORRHAGE.

By PERCY M. DAWSON.

EXPERIMENTS were performed on dogs. Varying quantities of blood were removed and replaced by equal amounts of various isotonic solutions infused intravenously.

The solutions used were normal saline (0.8% NaCl), Ringer No. 1 (CaCl_2 0.026%, KCl 0.03%), Ringer No. 2 (CaCl_2 0.01%, KCl 0.0075%, NaHCO_3 0.01%), and a mixture of one part milk and ten parts normal saline.

Omitting the cases of infusion with Ringer No. 1 (four out of six dogs died), the results obtained bore no apparent relation to the fluid infused either as regards the immediate effects on the pulse and respiration or on the subsequent changes in the blood.

Passing over the immediate effects, we will consider the changes in the blood. Blood examinations showed that immediately after hemorrhages there is a fall in the number of erythrocytes and in the amount of hæmoglobin (hemorrhagic fall), which is of course due to the removal of the blood from the animal. The number of erythrocytes and the amount of hæmoglobin, however, continue to decrease for from four to eight days (post-hemorrhagic fall).

With regard to the cause of the post-hemorrhagic fall, it has been shown that the freezing point of the serum was not altered by the hemorrhage, and hence we conclude that the post-hemorrhagic fall is not due to variations in the osmotic pressure of the plasma. It was also shown that during the period of the post-hemorrhagic fall the blood laked much more rapidly than in the normal animal. Hence we conclude that this fall is due to the low resistance of the circulating erythrocytes and consequently to an increased destruction of these elements.

Respecting the leucocytes we note (1) a marked hemorrhagic leucopenia in which the lymphocytes are proportionally less reduced than the polymorpho-nuclears and (2) a post-hemorrhagic leucocytosis without increase in the number of lymphocytes.

In conclusion, it is to be noted that as far as these experiments go, there appears to be no evidence that polymorpho-nuclears are formed from the lymphocytes, at any rate in the circulating blood.

THE SURVIVAL OF MAMMALIAN MUSCLE AFTER SOMATIC DEATH.

BY FREDERIC S. LEE.

UNDER the author's direction Messrs. Adler and Bulkley have been investigating the duration of the life of cross-striated muscle in mammals after the death of the individual. Cats and rabbits were the animals employed, and the muscles used were the deep-red soleus and the pale tibialis anticus. In each experiment the animal was killed, a particular muscle was excised and stimulated electrically at five-minute intervals, and the resulting contractions were recorded. The muscles survived several hours, the maximum in sixteen experiments being for the red muscle 14 hours and 37 minutes, and for the pale 12 hours and 20 minutes. The fact that the amount of nutritive sarcoplasm in comparison with contractile fibrillar substance is relatively larger in red than in white muscle fibres may perhaps account for the longer survival of the former. So far no constant difference in duration has been observed between the cat and the rabbit.

In both the soleus and the tibialis anticus the decrease of irritability was usually gradual, but occasionally in the latter there was a sudden fall at the end of about one hour, the irritability then continuing at a low ebb for hours, but with a gradual decline. The sudden fall may have been due to the early death of the white fibres, the later gradual decline to the longer survival of the red ones.

PHYSIOLOGICAL STUDIES ON MUCIN.

BY I. LEVIN.

MY experiments tend to show that thyroidectomy causes auto-intoxication from the accumulation of mucin in the blood. In the first series of experiments, a solution of mucin in one per cent solution of sodium carbonate was injected hypodermically into 8 normal rabbits and into 9 from which the thyroid had been removed. The former remained healthy, while of the latter only one survived. Some of the operated rabbits died within forty-eight hours after the injection, while they had previously survived the thyroidectomy from 11 to 25 days. The study of the influence of an intravenous

injection of a mucin solution on the blood pressure of a dog showed uniformly a fall of the blood pressure, even after both the vagi and the splanchnic nerves were cut (demonstration of tracings). Subsequent stimulation of the splanchnic increased the blood pressure. The fall was consequently due to the direct depressing action of mucin on the vasomotor centre in the medulla.

The conclusion to be drawn is that mucin accumulated in or introduced into the blood of a normal organism depresses the nervous system. This is not fatal to a normal organism, but decidedly is fatal to an animal deprived of the thyroid. Mucinæmia is the pathological state of an organism resulting from the absence of the thyroid function, though this conclusion does not exclude the possibility of other abnormalities arising from the same cause.

THE PROPORTION OF NITROGEN CAPABLE OF BEING SPLIT OFF FROM PROTEIDS BY THE ACTION OF ACIDS.

By R. H. CHITTENDEN (for VANDELL HENDERSON).

THE nitrogen contained in proteid substances apparently exists in the molecule in different kinds of combination, viz. as amid-nitrogen, monamino-, and diamino-nitrogen, etc. On boiling proteids with a mineral acid, a certain proportion of the nitrogen is split off as ammonia (amid-nitrogen), and can be obtained by suitable distillation of the fluid with magnesia. How far can the ammonia split off from proteids by this method be relied upon as a measure or index of the amount of amid-nitrogen present in the molecule?

In order to test this matter a large number of proteid substances were boiled for different periods of time with different strengths of hydrochloric and sulphuric acid, and the percentage of nitrogen split off as ammonia determined. While the results obtained agree with those of other observers, notably Nasse and Hausmann, in showing that the individual proteids differ widely in their yield of loosely combined nitrogen, thus suggesting differences of chemical constitution, the main feature of our results is that variations in the nature and strength of the acid employed, as well as variations in the period of heating, cause more or less noticeable variation in the yield of ammonia. In other words, the results obtained indicate that the amid-nitrogen of the proteid molecule cannot be determined accurately by this method.

THE PROPORTION OF BASIC NITROGEN YIELDED BY
ELASTIN ON DECOMPOSITION WITH HYDROCHLORIC
ACID.

By R. H. CHITTENDEN (for ALLAN C. EUSTIS).

THE lack of agreement between Bergh and Hedin, and Kossel and Kutscher in their study of the basic cleavage products of elastin led us to a study of the proportion of basic nitrogen split off from pure elastin by boiling for 100 hours with 20 per cent HCl and stannous chloride. Following the method adopted by E. Schulze, and determining the total nitrogen in the solution, the nitrogen in the form of ammonia, and the nitrogen in the phosphotungstic acid-precipitate, we have obtained very divergent results. In all, five distinct experiments were tried with the following results:

<i>Experiment.</i>	<i>Percentage of Nitrogen in form of organic bases.</i>
I.	0.86
II.	17.69
III.	15.57
IV.	6.50
V.	15.14

Our results led us to the conclusion that the method now in use for the separation of the hexone bases by phosphotungstic acid, and determination of the nitrogen therein, is unreliable for quantitative purposes, and that consequently results hitherto obtained by this method must be accepted with caution.

ON THE EXCRETION OF ALLANTOIN AND URIC ACID IN THE CAT. By L. B. MENDEL and E. W. BROWN.

ON ARTIFICIAL PARTHENOGENESIS. By J. LOEB.

Read by title.

ON A NEW THEORY OF THE PHYSIOLOGICAL EFFECTS OF AN ELECTRIC CURRENT. By J. LOEB.

Read by title.

A MODEL TO REPRESENT THE PRINCIPAL PATHWAYS IN THE NERVOUS SYSTEM. By G. P. CLARK.

xxxii *Proceedings of the American Physiological Society.*

APPARATUS FOR LABORATORY WORK BY LARGE CLASSES. By W. T. PORTER and others.

ON THE OCCURRENCE OF IODINE IN THE THYMUS AND THYROID GLANDS. By L. B. MENDEL.

This journal, 1900, iii, pp. 285-290.

ON THYMIN. By W. JONES.

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SOME FORMS OF APPARATUS USED IN THE COURSE OF PRACTICAL INSTRUCTION IN PHYSIOLOGY IN THE UNIVERSITY OF PENNSYLVANIA. By E. T. REICHERT.

A UNIVERSAL ARTIFICIAL RESPIRATION DEVICE. By E. T. REICHERT.

AN EXPERIMENT IN TELOGENY. By C. S. MINOT.

OBSERVATIONS ON THE DEGENERATION AND REGENERATION OF MOTOR AND SENSORY NERVE-ENDINGS IN VOLUNTARY MUSCLE. By G. C. HUBER.

This journal, 1900, iii, pp. 339-344.

A METHOD OF OBTAINING NUCLEIC ACID. By P. A. LEVENE.

Read by title.

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